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Vol. XXXII, Part II 1949

CORDYCEPS APHODII, A NEW SPECIES, ON PASTURE COCKCHAFER GRUBS

By J. MATHIESON, *Botany School, University of Melbourne*

(With Plates XII–XIV and 10 Text-figures)

INTRODUCTION

Cockchafer grubs have always caused a certain amount of injury to pastures in Victoria during the winter, but in recent years the damage done by the common Pasture Cockchafer, *Aphodius howitti* Hope. has become more severe, and in the winter of 1942, pastures were very seriously damaged in certain parts of Victoria. (Twentyman & Pescott, 1942). In general, subterranean clover pastures have been most damaged, the grubs destroying the whole plant, and the pasture coming to look as though it had been ploughed.

During the last two years, officers of the Department of Agriculture, who have studied the grubs in the field, have noticed that dead larvae in the soil often showed evidence of fungal attack. Before it is possible to assess the value of the fungus as a means of biological control, it is necessary to know its life history and the conditions which favour its development and spread in the field.

This paper deals only with the life history of the fungus, and gives some account of the biology of the parasite in the host.

LIFE HISTORY OF THE BEETLE

The beetles are dark brown, about half an inch long, with longitudinal parallel striations on their wing cases. They emerge in late January or early February and they gather together in myriads round exposed lights, and may also be found in enormous numbers in patches of dung in the fields. The cream-coloured eggs are about $\frac{1}{8}$ in. long and are laid in groups of thirty to forty in the ground in small earthern cells. After the larva emerges the sticky egg case adheres to the body as a shrivelled skin, but is soon lost as the grub moves through the soil. The length of the incubation period is not known accurately, but seems to be about three weeks.

The young larvae are only $\frac{1}{5}$ in. to $\frac{1}{6}$ in. of an inch long when they hatch, and the body remains white until they have their first meal of earth. The head is at first pale brown, but changes to dark brown within six to twelve hours. The rate at which they grow depends on the season; full-grown larvae have been found in April, but during the drought years 1943–5, when the rainfall was 24·05, 22·75 and 22·03 in. respectively (average annual rainfall 27·22 in.), the grubs were not full grown until well into May. When fully grown, the larvae are approximately 22 mm. long and 4–5 mm. broad. They are greyish white owing to the dark contents of the gut being visible through the transparent skin, and the head is dark brown to black;

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there is also a distinguishing pattern of hairs on the last segment of the abdomen.

At first, if the soil is dry, they feed only on roots, but, after the first autumn rains, the ground becomes soft enough for them to tunnel through it and they begin to feed on the foliage. They emerge at night and frequently carry a supply of leaves down into their tunnels where they remain during the daytime.

Most of the grubs continue feeding through the winter until about the end of August, when they pass into the non-feeding pre-pupal stage and grubs in this state are readily distinguished by their milky-white bodies. They remain in the soil in earthen cells until they pupate in October, finally emerging as the adult beetle at the end of January.

THE *CORDYCEPS*

Three different types of fungal growth were found on the dead larvae; the stromata of the *Cordyceps* and two stilboid forms, one of which was white and slimy, and the other at first white but rapidly developing areas of a waxy consistency which changed from bright yellow to an olive green. Although there was a strong possibility that these stilboid forms represented the conidial stage of the *Cordyceps*, they have been found to be parasitic on its stroma. The former is *Tilachlidium brachiatum* (Batsch.) Petch, and the latter belongs to the family Discellaceae of the Sphaeropsidales.

(1) *Morphology of the* Cordyceps *on the host*

(*a*) *Materials and methods*

Supplies of infected and uninfected larvae have been provided by Mr Twentyman of the Department of Agriculture, chiefly from Miner's Rest, near Ballarat, Victoria.

Stages in the development of the endosclerotium were studied from paraffin sections. The larvae were fixed in C.R.A.F. and in Carnoy's fixative, dehydrated, cleared in chloroform and embedded in paraffin. Sections were cut 7–10 μ thick and stained with Haidenhain's iron-alum haematoxylin and counter-stained with erythrosin.

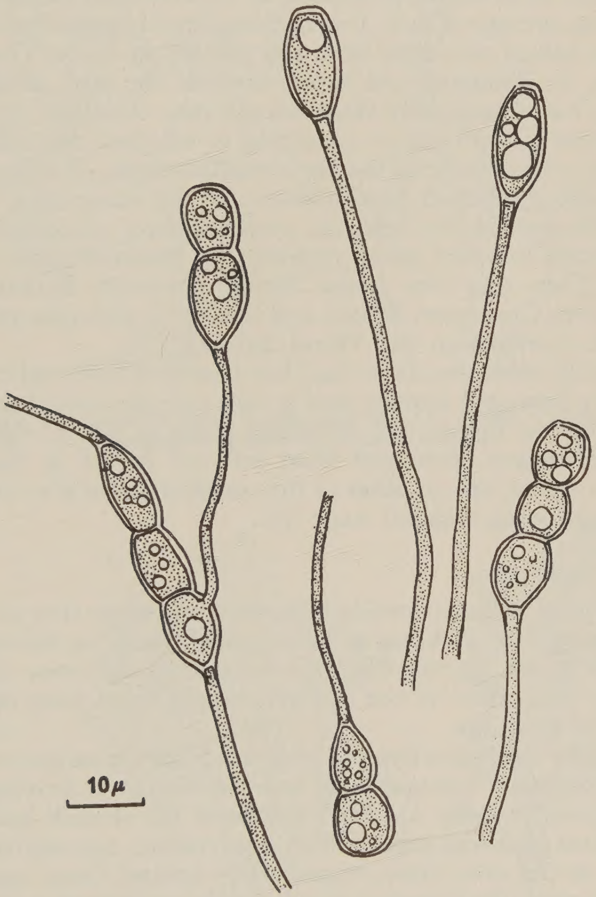
For the development and structure of the asexual and sexual stages of the fungus, both hand sections and paraffin sections were used. Temporary mounts were made in lacto-phenol and stained with cotton blue. Paraffin sections were prepared as described above, after the material was fixed in Fleming's weak fixative.

Dead and infected larvae were planted on the surface of non-sterile soil in large 5 in. Petri dishes, about ten larvae per dish, and incubated at room temperature and at 24° C.

The following account has been compiled from observations on larvae in the field, from naturally infected larvae which have been incubated in the laboratory, and from sections of infected grubs and stromata.

(*b*) *The Endosclerotium*

The external form of the larva remains unaltered after the formation of the endosclerotium, but the colour changes to either pinkish white or buff, the skin toughens and the body shrinks and becomes opaque. The interior is completely packed with a whitish mycelium, and the mass eventually becomes firm and often quite hard. The mycelium consists of extremely fine hyphae and gemmae. The gemmae are occasionally globose but usually oval with flattened ends; they may occur singly or in groups of two or three together, and may be terminal, intercalary or lateral to the hyphae. They are 7·5–15 μ long and 6–8 μ wide, and the hyphae are about 2 μ wide.



Text-fig. 1. Hyphae and gemmae from the endosclerotium.

(*c*) *The stromata*

As soon as the formation of the endosclerotium is complete and if environmental conditions are favourable, clubs begin to develop from the exterior of the body of the larva (Pl. XII, figs. 1, 2). These appear first as small cushions of mycelium on either side of the head and very quickly elongate to a length of about 1–1·4 cm. The length of the club varies according to the position of the grub in the ground, but in the field, the variation is very small, as most dead infected larvae are found in the upper half inch of soil.

Nevertheless, under certain conditions, clubs will grow longer than 1·4 cm. Infected larvae which were buried beneath one and a half inches of soil in the laboratory developed stromata up to 5 cm. in length, but they were very contorted and grew horizontally, so that none of them reached the surface. During growth they are positively phototropic, and it is conceivable that light controls the growth in length.

The number of stromata produced by a sclerotium varies from two to eleven with an average of five. Two of them usually grow from each side of the head, but others may arise from any part of the body. They are terete, about 2 mm. in diameter and taper towards the tip; usually they are unbranched, but occasionally short laterals may develop.

The stromata vary in colour according to whether they are completely sterile or bear one or other of the two conidial stages. It is unusual to find a sterile stroma developed from sclerotia in the laboratory, but often in specimens collected in the field the conidiophores of conidial stage (A) have been either knocked off or removed by predators and the stromata are naked. Then they are warm brown (between Buckthorn Brown, Dresden Brown, Cinnamon Brown and Russet*), with pale pinkish brown tips (between Avellaneous and Wood Brown).

Sections of the stromata show that they consist of a central core of brown longitudinally arranged hyphae and a very narrow outer layer where the hyphae are darker brown and arranged more or less at right angles to the inner. Stromata developed from infected larvae in the laboratory never remain sterile, one or other of the conidial stages always developing, the commonest being conidial stage (A).

(*d*) *Conidial stage* (*A*)

This stage may begin to develop from stromata when they are very small, often little more than cushions of mycelium showing on the outside of the sclerotia. It is not restricted to the stromata, and may develop from anywhere on the surface of the endosclerotium, even from the tips of the legs and along the hairs.

At first, coarse colourless hyphae grow out from the surface of the stroma. Each hypha branches monopodially and conidiophores develop at the ends of the branches (Text-fig. 2). At the extreme tip of each branch, several very short blunt phialides arise, which are hyaline, non-septate and about 6·25 μ long. At the same time, immediately behind these, and for a short distance back along the branch, a number of laterals develop and, becoming sickle-shaped, curve round the apex, at the same time producing many short blunt branches on their convex side. Colourless spores are budded off from the phialides until they completely fill the cavity enclosed by the outer sterile hyphae. The spores are oval, smooth-walled, unicellular bodies, and measure 5·0–7·5 μ long, and 2·0–2·5 μ broad. The sterile hyphae are 3·75 μ wide, and markedly tuberculate. At first they are colourless, but at maturity become buff-coloured, so that the whole stroma, when covered

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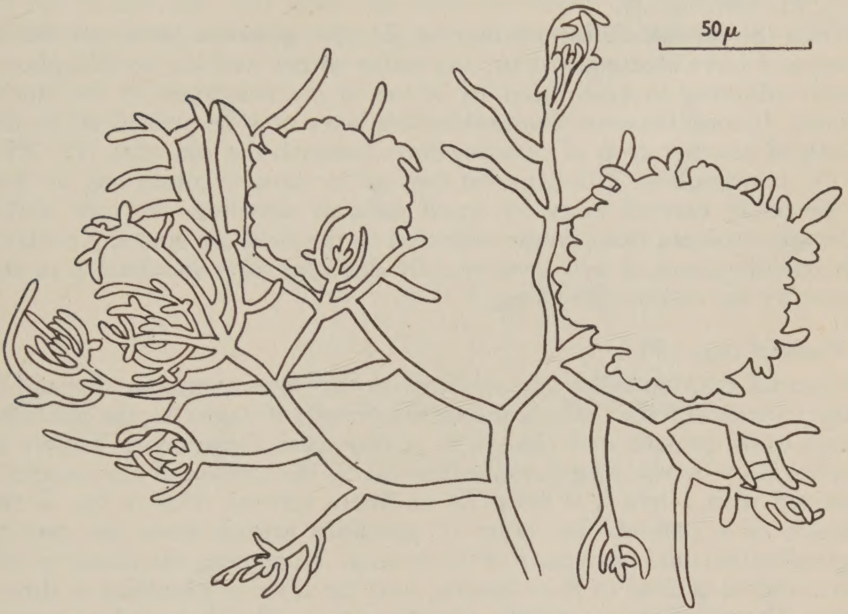
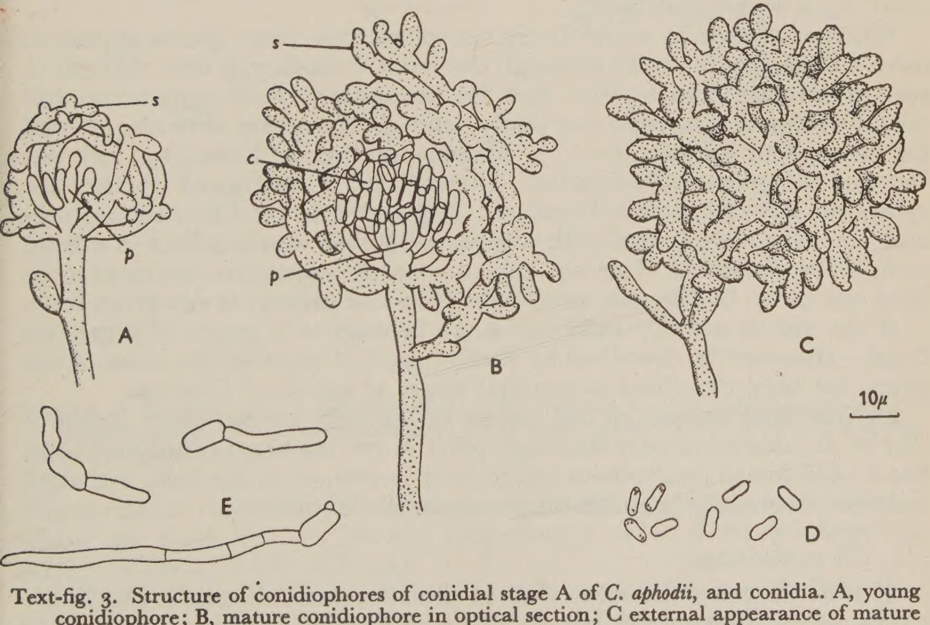
* The colours quoted are from Ridgway's Colour Standards.



Text-fig. 2. Conidiophores of conidial stage A, of *C. aphodii*. The mature conidiophores are only shown in outline.



Text-fig. 3. Structure of conidiophores of conidial stage A of *C. aphodii*, and conidia. A, young conidiophore; B, mature conidiophore in optical section; C external appearance of mature conidiophore; D, conidia; E, germinating conidia. *c*, conidia; *p*, phialides; *s*, sterile hyphae.

with these conidiophores, is Ochraceous Buff to Ochraceous Tawny (Text-fig. 3; Pl. XII, fig. 4).

When the conidiophores are mature, i.e. the spherical heads are full of spores and have changed colour, the stalks wither and the conidiophores remain adhering to each other by virtue of the roughness of the sterile hyphae. If conditions are favourable, this layer may be pushed off by the growth of another crop of conidiophores beneath the old ones (Pl. XII, fig. 5). It is doubtful if they persist thus under natural conditions, as they are probably carried away by small animals crawling over the clubs. Although stromata from larvae collected in the field are sparsely covered with conidiophores, a new crop rapidly develops after incubation in the laboratory for two to three days.

(*e*) *Conidial stage* (*B*)

A second conidial stage may develop on the same stromata. Instead of being covered with the whitish, pubescent growth of stage (A), the stromata remain quite smooth and change in colour from Ochraceous Tawny to between Raw Sienna and Antique Brown. If the surface of the stroma is examined with a lens it is found to be finely velvety. This is due to the presence of a palisade-like layer of phialides arising from the central longitudinally running hyphae of the stroma. In section, the colour of the central region is close to Raw Sienna, and the layer of phialides is almost Russet. The phialides are simple, smooth and are slightly broader towards the apex; each one terminates in a fine sterigma. They are 13·0 μ long and 2–3 μ broad at their greatest width. Each sterigma bears a very slender, pip-shaped spore. The spores are hyaline, smooth and measure 5·0 μ long and 1·25 μ wide (Text-fig. 4).

When examined in water under the microscope these spores appear to have a thick hyaline wall, although the outer boundary is very difficult to see. When stained with cotton blue the protoplasm of the spore stains, but the wall remains colourless and badly defined. The spores were also stained for twenty-four hours with 0·1 % aqueous solution of Ruthenium red, but the wall still remained colourless. It is difficult to determine whether the spores are dry or coated with mucus. If spores were produced successively and the walls were coated with mucus, the spores would collect in a head as in a *Cephalosporium*. It is not known whether a sterigma produces more than one spore, but there is never more than one present at any given time.

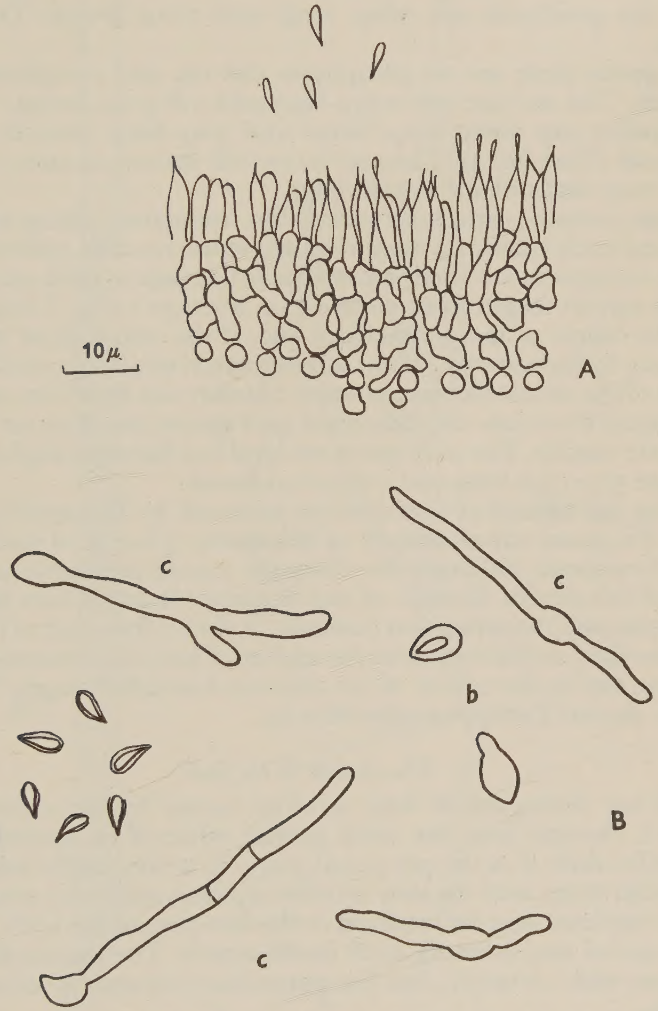
If the spores are dry then this stage belongs to a genus of imperfect fungi—*Hymenostilbe*, described by Petch (1931). A number of species of this genus has been described as conidial stages of species of *Cordyceps*.

Further observations on the fungus in the field are necessary before it can be decided what part this stage plays in the life history, but only once has it been found on stromata which have developed in the field, although it occurs frequently on stromata grown in the laboratory.

(*f*) *The perfect stage*

This develops on the same club as the two conidial stages. The fertile part is at first intercalary, but may become terminal if the pointed sterile apex develops a few perithecia. It is ovoid to cylindrical and is usually

3–4 mm. long and about 1·5 mm. broad. The colour is the same as that of the rest of the club, and when this stage developed on incubated sclerotia in the laboratory, conidial stage (B) was often present between the ostioles.



Text-fig. 4. Conidial stage B of *C. aphodii*. A, T.S. of stroma showing phialides and conidia; B, germinating conidia. *a*, conidia before germination; *b*, conidia swollen prior to germination; *c*, germinating conidia.

The surface of the fertile part is punctate and slightly papillate, the ostioles, which are dark reddish brown, projecting a short distance above the surface of the club (Pl. XII, figs. 6, 7).

Sections of the fertile head show that it consists of three well-marked layers: (1) a central columella where the hyphae are arranged longitudinally, (2) a wide layer surrounding the columella which forms a spongy matrix in which the perithecia are embedded, and (3) a narrow outer layer of short hyphae arranged at right angles to the main axis. The perithecia are arranged obliquely to the columella and most of them have upwardly curved necks (Pl. XIII, figs. 1–4). They are flask-shaped and have a dark brown wall consisting of several rows of cells. The average measurements of the perithecia are 360 μ long and 110 μ broad. The wall is 6·5 μ thick.

In this genus there are no paraphyses and the asci completely fill the perithecium. The asci are 200–225 μ long and 3·8–5·0 μ broad. Each has a thick hyaline cap about 8·0 μ broad and 5·0 μ long, crossed by a very narrow canal (Text-fig. 5). The asci do not all mature at once, and spore discharge may continue for several days.

The ascus contains eight long thread-like ascospores which are slightly coiled round each other. As they mature, septa become visible and just before the ascospores are shed, constrictions develop at each septum. The ascospores vary in length from 90 to 151 μ, average 116 μ. They are shed through the ostiole of the perithecium and, if the atmosphere is dry, are carried away by air currents. If the atmosphere is moist, they collect round the mouth of the ostiole like cotton-wool. As they are shed they may break up into lengths if not into the individual part spores, but if water is present they separate readily. The part spores are oval but flattened slightly at each end and are 5·5–7·5 μ long and 1·25–2·0 μ broad.

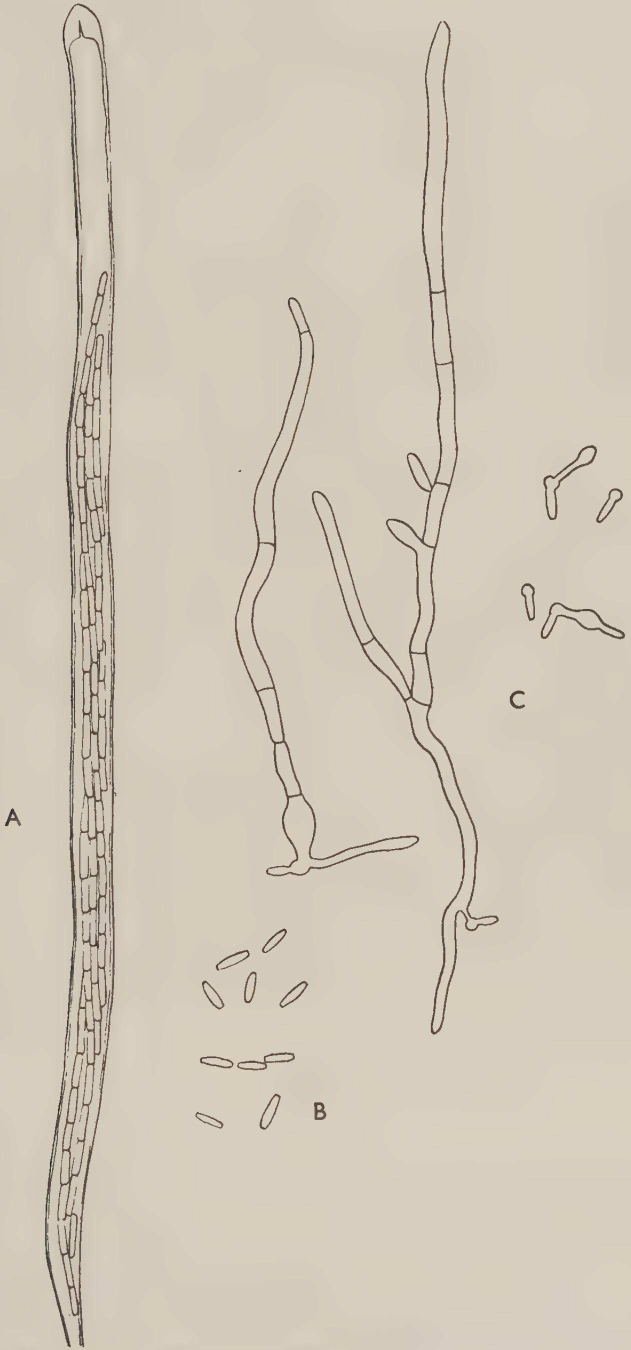
Following the scheme of classification proposed by Kobayasi (1941) for species of this genus, the characters of this species place it at once into the subgenus *Eucordyceps*, although the obliquely placed perithecia are rare in members of this group. Because of this character it comes into the section *Cremastocarpon*, and the subsection *Carnosae*. It differs from any of the species already described in this section in the species of host, the dimensions of the perfect stage and in the nature of the associated conidial stages. Therefore it has been named **Cordyceps aphodii** n.sp.

(2) *The disease in the field*

Infected are distinguished from healthy larvae by the colour of the body, which changes from the usual greyish white of an actively feeding grub, or milk-white if in the pre-pupal stage, to a faint buffy yellow often with a pinkish tinge, and the skin becomes opaque and more wrinkled. At first these symptoms may be localized in the fore-part of the body, but they gradually spread and intensify until death occurs. The incubation period is not known with certainty, but lies somewhere between a fortnight and two months.

Infection may take place any time from hatching in February until August, when the grubs have all passed into the pre-pupal stage. After this, no grubs in the early stages of infection were ever found, which suggests that either the grubs in this condition are resistant to infection, or since they are inactive, are not exposed to it.

From April to August conidial stage A is always found on stromata in the field and develops abundantly on grubs incubated in the laboratory, but conidial stage B, though developing freely in the laboratory, was only found once in the field. The perfect stage began to develop on incubated



Text-fig. 5. A, ascus containing mature ascospores; B, part spores; C, germinating part spores.

grubs at the beginning of August and by October development was complete; at the same time, mature perithecia were found in the field.

The significance of the ascospores in the life history of the parasite is obscure, unless they remain viable for some months in the soil, because at the time they are shed the grubs are all in the pre-pupal stage and unlikely to be exposed to infection. It is possible, though not very likely, that the ascospores germinate and grow saprophytically in the soil until the young grubs hatch out in the autumn.

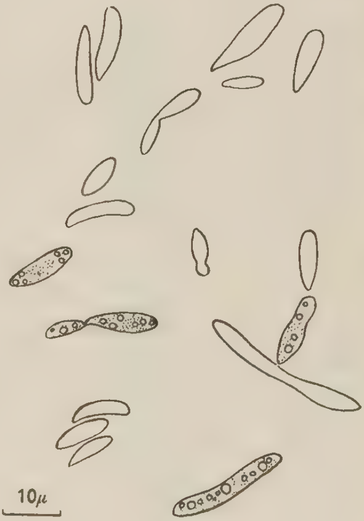
During the summer months the clubs shrivel, but they appear to be able to revive again under moist conditions. One specimen, which had been dried for twelve months, was planted again on moist soil; the clubs revived and added 4 mm. to their length, as well as developing conidial stage A.

In February 1947, after one and half inches of rain, old stromata were found which had revived and produced a new crop of spores of stage A. Such stromata may provide the source of infection for the new season's larvae.

(3) *The development of the parasite in the host*

The method of entry of the parasite into the host is unknown, but examination of sections of infected larvae suggests it does not gain entrance through the alimentary canal as it is never present in any part of the gut, nor in the cells of its lining. The other means of entry are through the skin, or the tracheal tubes. Since it has not been possible to infect larvae with the fungus artificially, it has been necessary to select individuals at random for sectioning, as in the initial stages of infection diseased are indistinguishable from healthy larvae.

The fungus is present in the host in the form of hyphal bodies (Text-fig. 6). These are oval-fusiform yeast-like cells, which are usually unicellular but occasionally once-septate, and they reproduce by budding. A number of apparently healthy larvae were sectioned and in several of them these hyphal bodies were present, but in none of them was there any sign of mycelium or any indication of how the parasite entered the body of the host.



Text-fig. 6. Hyphal bodies from an infected larva.

The larva of *Aphodius* has a large and much-coiled gut which is surrounded by a haemocoele in which the rest of the tissues and organs of the body are situated. The head is occupied very largely with striated muscle which is also well developed throughout the rest of the body. Early in life the fat body is small, but it gradually increases in size until at the pre-pupal stage it occupies almost the whole of the body cavity. The spiracles are situated along both sides of the body, and each leads into a cavity from which radiate the tracheal tubes.

In the earliest stages of infection which have been examined, unicellular hyphal bodies were present throughout the haemocoele, and on the walls of all the organs in contact with it. They were clustered in large numbers round the tracheal tubes especially (Pl. XIV, fig. 2), and round the muscles in the head. They were also present on the inside of the epidermis, round the fat body, the body muscles, the Malpighian tubes and on the outer surface of the wall of the gut. Such is the distribution at the first outward signs of infection, although by this time the numbers of hyphal bodies present have increased enormously. As the symptoms spread to all parts of the body a change takes place in the character of the parasite; instead of the daughter cells separating as before, they remain attached to the parent and small chains of cells develop which come to occupy the whole body cavity. They are clustered in tremendous numbers round the gut which by this time is considerably compressed, and are also present in and between the fat body (Pl. XIV, fig. 3), but the cuticle and muscles are still intact. It is at this stage that death occurs. The body of the host is still quite soft and further development of the parasite takes place before the formation of the mature endosclerotium. The chains of cells give rise to very fine hyphae which penetrate the skin, the muscles and the fat body, so that although these structures are recognizable in the body of the larva, they are completely permeated by the fungus. The hyphal chains therefore become the typical gemmae of the sclerotium.

Hyphal bodies similar to those found in *Aphodius howitti* have been described by de Bary (1884) for *Cordyceps militaris*, and by Petitt (1895), for *C. clavulata* in scale insects. They both described them as yeast-like bodies, and Petitt also describes some in *Lecanium fletcheri* which were larger and once or twice septate, possibly merely the same bodies at a later stage of development. De Bary states that the part spores germinate on the skin of the caterpillar in *Cordyceps militaris*, which they penetrate at any point on the surface and develop a mycelium of stout hyphae which penetrate the muscles and fat body. Mycelial growth then stops and instead short 'cylinder-gonidia' are cut off from the ends and sides of the hyphae and set free into the blood stream. These are the hyphal bodies. No trace has been found in *C. aphodii* of any initial mycelium from which the hyphal bodies develop, nor of the means of entry of the fungus into the host.

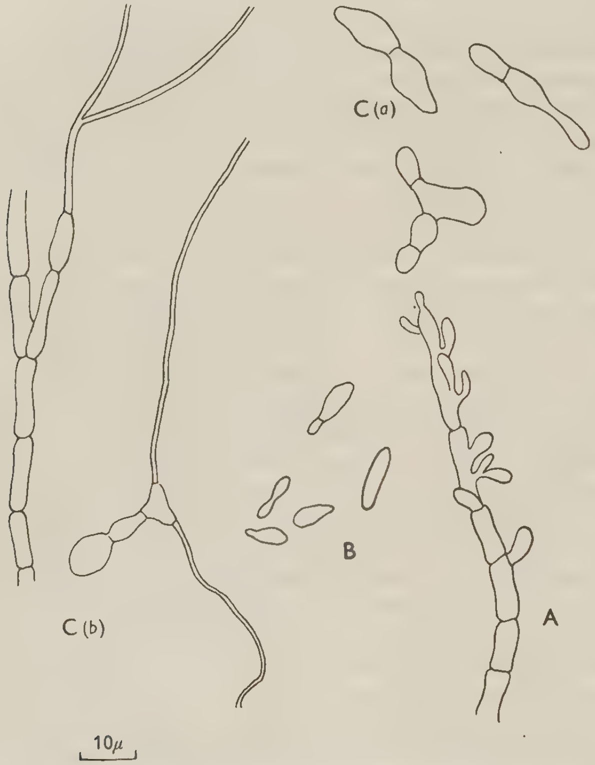
(4) *The* Cordyceps *in artificial culture*

(*a*) *Materials and methods*

Sterile cover-slips were coated with a thin film of sterile, filtered, melted agar. A spore suspension was made in water and a few drops spread over the surface of the agar. The cover-slips were then incubated in sterile Petri dishes lined with moist filter-paper. The cover-slips were examined at intervals aseptically through the back for germinating spores. Single spores were then cut from the agar using a cutter which fits over the low-power objective and has the diameter of the low-power field. The blocks of agar bearing single germinated spores were then transferred to agar plates.

Potato-dextrose agar was found to be the best medium for germination of spores and subsequent growth of the colony.

Cultures were also made on the larvae themselves. They were killed by exposing to chloroform vapour, then placed on a pad of moist cotton-wool in a test-tube and autoclaved for fifteen minutes at one atmosphere. Cereal grains were used in an attempt to induce the formation of the perfect stage. Rice, barley and wheat grains were saturated with water and autoclaved in flasks.



Text-fig. 7. A, hyphal bodies developing on a hypha in liquid medium. B, detached hyphal bodies. C (*a*), chains of cells; (*b*), types of mycelium developed in culture.

(*b*) *Germination of ascospores*

Fertile clubs were suspended over sterile cover-slips inside a sterile Petri dish overnight and a heavy deposit of part spores was obtained. A spore suspension was made in sterile water and sown on thin films of nutrient and potato-dextrose agar. Germination always occurred within three days at 24° C. A swelling was first produced at one end of the spore and later a germ tube developed from this (Text-fig. 5C). Growth ceased at this stage on nutrient agar, but on potato-dextrose normal germ tubes were produced, very much broader than the part spore, and a mycelium was developed. Single germinated part spores were transferred to plates of potato-dextrose agar and incubated at 24° C.

The fungus is particularly slow-growing and the colony produced is very restricted. Visible growth from a single spore appears after seven to ten days as a tiny mound of rather moist-looking mycelium which is faintly tinged with pink. Growth continues at approximately the same rate both vertically and horizontally. Most of the mycelium is submerged and this submerged growth gradually builds up until a convoluted mound of mycelium is produced. A very short, whitish pubescent growth of aerial hyphae develops on the surface of the mound, and surrounding the latter is a narrow ring of submerged mycelium. The colour of the colony is between Ecru Drab and Light Cinnamon Drab, and the reverse varies from between Vinaceous Buff and Avellaneous to Deep Brownish Drab and Dusky Drab. At this stage, i.e. eighteen to twenty-one days, conidial stage A may develop throughout the aerial hyphae although the period which elapses before this stage is produced varies considerably. One factor seems to be the depth of medium (and therefore presumably the maturity of the colony) as it appears more quickly in Petri dish cultures than on slopes.

On slopes after six weeks there may still be no sign of conidial stage A and the colony deepens in colour to Pale Purple Drab while the medium is Light Cinnamon Drab. At this stage, parts of the surface of the colony may change in colour to between Deep Colonial Buff and Chamois. Eventually, spherical conidiophores of stage A are produced usually on localized parts of the surface of the mound and the colour then changes to between Buckthorn Brown and Antique Brown. Stromata develop five weeks after inoculation of a plate, but on a slope the time is correspondingly longer. The number varies from one to ten, and they are either sterile or bear the spherical heads of conidial stage A, but never stage B nor the perfect stage. The colony is now mature, and no further growth takes place even when the entire growth is transferred to fresh medium. The maximum diameter is about 15 mm., and the colour of the reverse has changed to between Saccardo's Umber and Bister.

Microscopic examination of a young colony shows that it consists of a mass of coarse hyphae closely septated, from 3 to 4 μ wide, and with some very fine branches about 1 μ wide. In addition, there are short lengths of hyphae consisting of two to four irregularly swollen cells which develop as laterals on the main hyphae and then become detached; these may in turn give rise to long fine hyphae. The whole mass may be up to 5 mm. thick, and is of a leathery consistency. Later, conidial stage (A) develops amongst the aerial hyphae in the same way as on the stromata or the sclerotia.

(*c*) *Germination of spores of conidial stage A*

The heads were crushed in water so that the spores were liberated. These were then sown on potato-dextrose agar according to the method described above for isolation of single spores. Germination was extremely slow even at 24° C. In one case germination occurred after five days and in others between seven and nine days. The spores swell considerably in size, and germinate by a single germ tube or by one from each end (Text-fig. 3). The hyphae are coarse and closely septate. The colony produced is identical with the colony produced by part spores.

(*d*) *Germination of spores of conidial stage B*

Germination occurred on potato-dextrose agar after four days at 24° C. The spores swelled enormously, becoming quite oval, and developed either one or two germ tubes, one from each end of the spore (Text-fig. 4). The hyphae are coarse and closely septated, and the colony formed is identical with that from part spores.

(*e*) *Growth from hyphal bodies*

It was found (see section 3) that the blood of living larvae which were suspected of being infected was swarming with yeast-like hyphal bodies. Cultures were made from these hyphal bodies to compare the type of colony produced with that from part spores. The skin of a living infected larva was pierced with a needle, and the liquid which exuded was streaked with a sterile loop over the surface of a potato-dextrose agar plate and incubated at 24° C. Growth was very slow and was hardly visible after a week. Under the microscope it was obvious that the hyphal bodies had grown, but instead of budding as they do inside the larva they had developed short lengths of swollen bead-like hyphae, some of which had produced fine hyphae-like germ tubes from either end. In others, these fine hyphae had become septate and developed another row of swollen cells on the other end. As growth continued, a colony which was similar in character to that from part spores was formed, finally producing conidia of conidial stage A.

(*f*) *Growth on other media*

(i) *On sterilized larvae.* Several larvae were inoculated with a suspension of part spores. The skin was lightly pricked with a needle, and a drop of liquid exuded. The part spores were sown in this liquid. After twelve days at 24° C. the bodies of the larvae had become pink and a whitish pubescence was apparent at the point of inoculation. They finally became buff-coloured like the sclerotia collected in the field, and developed stromata and conidial stage A (Pl. XIV, fig. 3).

(ii) *On cereal grains.* Kobayasi (1941) obtained fertile stromata of *C. militaris* on sterilized moistened rice grains. An attempt was made to do the same with *C. aphodii*, using wheat, barley and rice grains. Flasks of each were inoculated with a suspension of ascospores, and after sixteen days at 24° C. the rice grains began to show a pinkish discoloration and a very slight whitish down could be seen round the inoculum. Between four and six months after inoculation the rice grains were completely permeated with mycelium, and small slender stromata had formed, which were covered with conidial stage A, but there was no sign of perithecia. After six months the barley and wheat grains were also permeated with mycelium, but only two small stromata had formed.

(iii) *On liquid potato dextrose.* Since growth of hyphal bodies on a solid medium gave rise to a mycelium and not to more hyphal bodies it was thought that since development takes place in a liquid medium, the blood, this type of growth may be a natural response to the type of medium. Therefore liquid potato dextrose, having the same composition as the potato-dextrose agar, was inoculated with spores of conidial stage A. The pH of the blood was found to be between 6·0 and 7·0, so the medium was adjusted to both pH 6 and 7, and both sets were sown with spores. The spores germinated, and the colonies were at first submerged, developing as small spherical masses of mycelium. Later some of these reached the surface of the liquid and assumed the usual form of a colony on potato-dextrose agar. They were found to consist of hyphae and hyphal segments like the colony on agar, but large numbers of hyphal bodies similar in shape and size to those found in the host were also present in the medium. They are produced in clusters along the coarse hyphae and being quite narrow at their point of origin are easily detached. They then continue to grow by budding.

(5) *Infection experiments*

Having isolated and grown the parasite in pure culture, the next step was to reinfect the host larvae. Unfortunately all efforts to do this have failed, owing mainly to the fact that handling of any sort, however gentle, seems to damage the larvae, and there is a very high mortality. It is possible, also, that the conditions under which the larvae were kept in the laboratory were unfavourable, but the fact remains that from two experiments, using over 1000 living larvae, so few survived that any results were useless. The death of the larvae was obviously not due to the parasite as after death they usually turned black and disintegrated, instead of forming a sclerotium, and death was always too rapid.

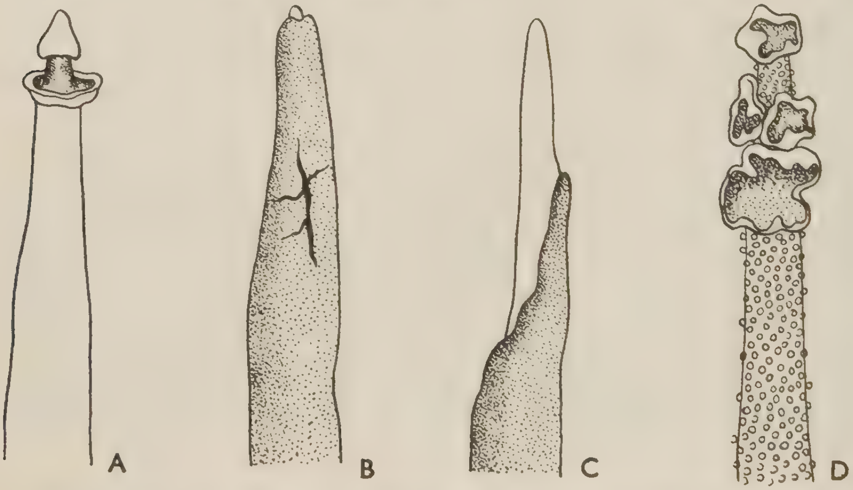
OTHER FUNGI FOUND ON THE SCLEROTIA IN THE FIELD

In addition to the *Cordyceps*, two other fungi are associated with the sclerotia in the field. Both of these may occur as stilboid outgrowths from the sclerotium, and at first it was thought that they may be the conidial stage or stages of the *Cordyceps*. It has been shown, however, that they are both parasitic on the sclerotium or on the actual stromata themselves. As well as occurring in the field, sclerotia incubated in the laboratory on moist soil invariably develop one or another, and sometimes both, of these fungi.

(1) *A species of the family Discellaceae*

This may first appear as small tufts of white hyphae arising from the sclerotium. These rapidly elongate and form white clavae up to 2 cm. but usually 0·5–1·0 cm. high, which may or may not be branched, and may remain cylindrical or spread out fan-wise (Pl. XIV, fig. 1). Sooner or later during their development a yellow waxy layer develops in various positions on the club. It may completely envelop the white clava leaving a tiny white tip projecting at the apex, or it may develop in localized areas. Often it encircles the clava near the apex and at its lower boundary the white hyphae of the clava grow outwards forming an upturned flange with the yellow layer lining its inner and uppermost surface; then at its upper edge another smaller downwardly directed flange develops and the clava ends in a small white pointed tip. The whole structure resembles a minaret.

Many variations of this form may occur. Sometimes the clava is short and stumpy and the waxy layer covers the whole structure except for a white cup at the base and a white cap at the apex. As well as developing on its own clavae, it may form on the stromata of the *Cordyceps*. Here it takes the form of cup-shaped sporodochia with crenulate edges, which develop laterally on the clavae. The outer surface of the cup is white and the inner is lined with the yellow waxy layer (Text-fig. 8, Pl. XIV, fig. 2). As the clavae age, the yellow layer may alter in colour to a deep olive green.

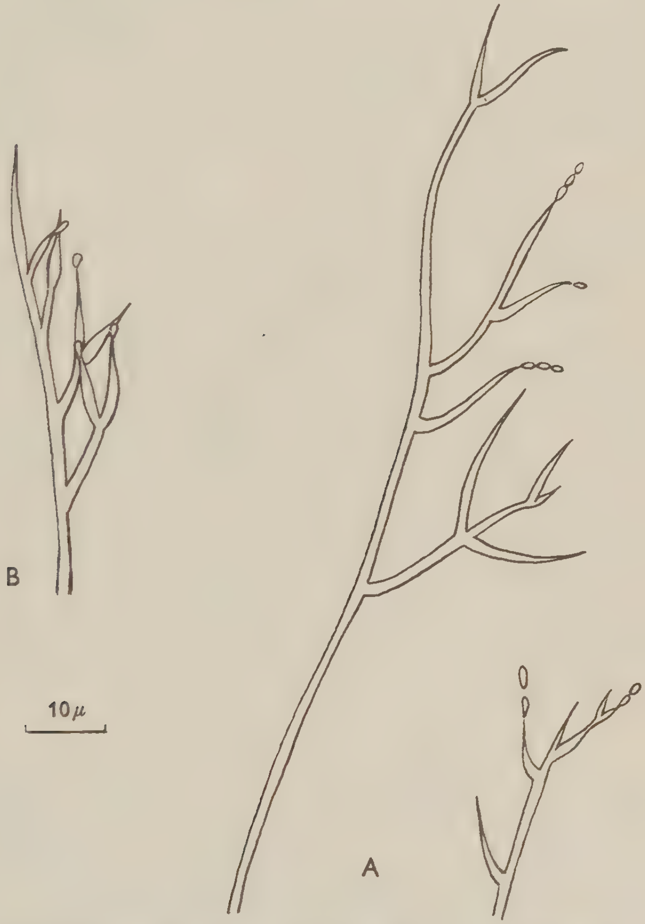


Text-fig. 8. Types of fruiting bodies of parasite (1). × 4. A–C, the dotted part represents the yellow waxy areas; D, open 'pycnidia' of this form on a stroma of *C. aphodii* covered with conidial stage A.

The clava consists of a central region of longitudinally running hyphae, and in the white part of the clava the outer hyphae curve outwards and develop conidiophores at their apices. These arise from a main hypha and may be unbranched, dichotomously or trichotomously branched (Text-fig. 9A). They are slender and about the same width as the hyphae, but taper towards the tips. Conidia are produced in chains at the tips of the branches and are smooth, hyaline, oval in shape, and measure 1·25 μ long, and 2·5–3·0 μ broad.

In the yellow waxy areas the conidiophores form a very compact palisade layer at right angles to the central core. The conidiophores resemble those in the white areas but are not so lax and the phialides are more regular in their formation (Text-fig. 9B). The lateral branches all develop on one side of the main hypha, and are usually bifurcate but may be unbranched. The spores are smooth, hyaline and globose, measuring 1·25–1·9 μ in diameter. They are produced in enormous numbers and accumulate in a thick layer above the conidiophores. At first the whole mass is enveloped in mucus, but this gradually dries out and the spore layer becomes waxy.

Since the spores produced by these areas differed in shape and in the method of production, i.e. one in chains and the other in masses, it seemed possible that they may belong to different fungi, and that one was parasitizing the other. Spores from each area were sown in the manner described above for single spore isolation. After two days, spores from the white area had germinated and produced long germ tubes. Those from the yellow area had just begun to germinate. A number of single germinated spores were isolated and plated out on potato-dextrose agar. Exactly the same type of colony was produced by each type of spore.



Text-fig. 9. Conidiophores of parasite (1) (Discellaceae). A, conidiophores from white clava; B, conidiophores from the yellow waxy area.

At first a very short white aerial mycelium develops on the surface as the submerged hyphae spread out from the inoculum. This consists almost entirely of conidiophores and conidia of the 'white' variety, but in some of the conidiophores the spore chains have collapsed and the spores adhere in a spherical mass to the tips of the phialides like a *Cephalosporium*. Gradually small circular areas develop which are cream-coloured and covered with liquid. These may coalesce, forming a more or less continuous cover, and as they mature, darken in colour to bright maize yellow and finally become waxy. These areas consist of conidiophores of the type found in the yellow waxy areas under natural conditions. At about one centimetre from the centre of the colony a ring of clavae develop which at first are white but eventually are covered entirely or in part with the yellow waxy coat so typical of this fungus. Growth then continues horizontally as before with mixed white and yellow areas, then another centimetre further away from the inoculum another ring of clavae develops (Pl. XIV, fig. 3). On potato-dextrose agar the diameter of the mature colony is 2·6–2·7 cm. The reverse is brown.

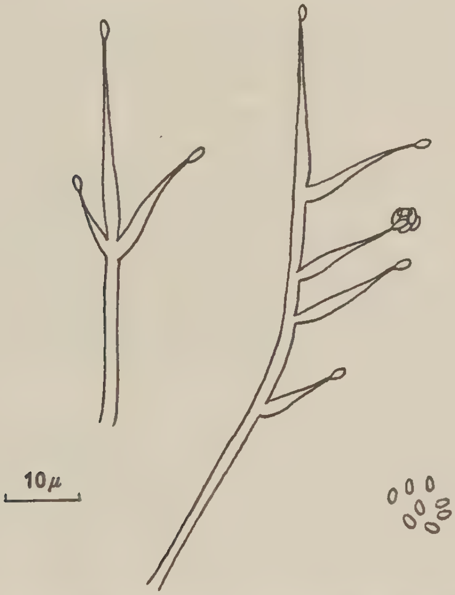
The order of the Fungi Imperfecti into which this form is placed would vary according to which particular form of sporing body one regards as typical. If the open cup-shaped structures, lined with a thick moist mass of conidia, which develop on the stromata of *Cordyceps* and on the endosclerotia, were the only fruiting bodies developed, the fungus would be classified in the Discellaceae, a section of the subfamily Patellinae.

Sometimes only the white and yellow synnemata are developed and then it would be better described as a stipitate *Tubercularia*. In fact, Petch (1939) describes a fungus on sclerotia of a *Cordyceps* on 'white grubs' which has similar white and yellow clavae although with conidiophores of *Gliosporium* and calls it a stipitate *Tubercularia*. The most constant features are the type of conidiophore and the slimy nature of the spores, and it would seem more reasonable to classify the fungus on such characters rather than on the form of the fruiting structure when the latter is so variable. But according to the present system of classification the most convenient systematic position, i.e. one which would facilitate subsequent identification, is in the Discellaceae, subfamily Patellinae, as this is the form in which it is found most frequently under natural conditions.

(2) *Tilachlidium brachiatum* (Batsch.) Petch

The second fungus also develops from the sclerotium as white tufts of hyphae which eventually elongate forming groups of white clavae, up to 7 mm. in height. Each clava bears numerous fine synnematal branches which give a coralloid appearance to the whole growth. The clava and its branches are covered with *Cephalosporium* conidiophores; each head is enveloped in a drop of mucus, and as the heads develop these drops frequently coalesce and the whole clava is covered with mucus and becomes cream-coloured (Pl. XIV, fig. 4). The phialides are simple and arise as laterals from a main hypha, sometimes two from one level. They are slender, slightly inflated towards the base and tapered to a fine tip. The spores are smooth, hyaline and oval, 0·75–3·0 μ long, and 0·5–1·75 μ wide (Text-fig. 10).

Single spore cultures were grown on potato-dextrose agar. After twelve days the colony measures about 3 cm. in diameter. At first it is white but later ranges from Light Buff to Pale Pinkish Buff. The surface is covered with hundreds of tiny synnemata which vary in height from 0·4 to 1·0 mm., and some of them bear very fine branches. In addition to these small synnemata, which develop over the entire colony, larger ones may develop in groups round the inoculum. These may reach a height of 7 mm. and like the smaller ones bear many short slender branches giving the synnemata a coralloid appearance. If the surface of the colony is examined under the microscope the globular heads of conidia are easily visible entirely covering the larger, but usually only partially covering the smaller synnemata. The mucus on the spores is not so conspicuous as it is when the fungus is growing under natural conditions, i.e. on the *Cordyceps* sclerotium, where each head is enveloped in a globule of mucus, but there is still sufficient to make them adhere, and form a head. Individual heads measure between 6 and 7 μ. The colony is brown in reverse.



Text-fig. 10. Conidiophores and conidia of *Tilachlidium brachiatum* (Batsch.) Petch.

Tilachlidium brachiatum (Batsch.) Petch, is a member of the Stilbaceae. As Petch (1945) has emphasized, *Tilachlidium* is not a coremial *Cephalosporium*, as there is a central core of agglutinated hyphae, the outer branches of which bear the conidia, instead of the tip of each hypha of the clava ending in a conidiophore. Petch found that the form known as *Isaria brachiata* (Batsch) Schum. was really a *Tilachlidium*, and named it *T. brachiatum* (Batsch.) Petch. He also included the type species *T. pinnatum* Preuss and *T. subulatum* A. L. Sm. under *T. brachiatum* as simple forms of the latter. It occurs on a variety of substrata, including leaves, roots, and decaying agarics, and is parasitic on *Stilbum tomentosum* and certain entomogenous fungi. Petch states that 'the clavae occur in clusters and are white to pale brown, simple or branched'. The whole clava is covered with *Cephalosporium* conidiophores, which are perpendicular to the main axis.

The conidia are described as 'cylindrical or narrow oval, hyaline, 2–7 (usually 3–6) × 0·75–1·5 μ'. These dimensions are different from those of the form on *Cordyceps aphodii*, but the dimensions appear to vary considerably within the species, for Petch gives the size of the conidia of the form on *Stilbum tomentosum* as 5–12 × 2–4 μ, and of a specimen of *Isaria brachiata* on a leaf hopper as 2–4 × 0·75–1·0 μ. Since these have all been included by Petch in this species, the form on *Cordyceps aphodii* has also been called *Tilachlidium brachiatum* (Batsch.) Petch.

DISCUSSION

In his monograph on the genus *Cordyceps*, Kobayasi (1941) regards the relationship between the conidial stage and the perfect stage as certain even if the conidia are merely produced on the perfect stroma or on a mycelium covering the endosclerotium. A relationship on these grounds was claimed by Tulasne (1853) for *Isaria farinosa* and *Cordyceps militaris*, although he did grow the fungus from part spores and Petch (1936), after examining his figures of conidiophores which develop from these colonies, considers that they are those of the true conidial stage which is a *Cephalosporium*, and not those of *Isaria farinosa* as Tulasne maintained.

In four species only of *Cordyceps* has the relationship between the conidial and the perfect stage been established in culture. *C. militaris* was shown by Petitt (1895) and by Petch (1936) to develop *Cephalosporium* conidiophores in culture; *Cordyceps clavulata*, for which Petitt (1895) obtained an asexual stage in cultures of hyphal bodies which corresponded with that formed under natural conditions, and has since been described by Petch (1933) as *Hirsutella lecaniicola* (Jaap) Petch; and Möller (1901) obtained *Cephalosporium* conidiophores from ascospore cultures of *Cordyceps rubra* and *C. submilitaris* (*C. martialis*). It has not been possible to obtain perithecia in culture from any of the spores of *C. aphodii*, but as spores from both of the suspected conidial stages produce a colony which is similar to that produced by the part spores, and they all develop the same type of conidia, it is very probable that they are all forms of the same fungus.

As far as I am aware, conidiophores similar to those of conidial stage A have never been described before, either as a conidial stage of *Cordyceps*, or in the Fungi Imperfecti. They bear a superficial resemblance to the bulbils produced by many genera of fungi, which, when they are not connected with a perfect stage, Hotson (1912, 1917) has described under the form genus *Papulaspora*. Although these bulbils are reproductive bodies, and consist of a mass of homogeneous or heterogeneous cells, one or all of which are capable of growth or germination, true conidia are never produced, nor anything approaching the complex structure of the head in stage A of this *Cordyceps*. *Papulaspora* has not been recorded on insects, but *Aegerita webberi*, which is similar in many respects, has been described by Fawcett (1910) on a scale insect and also by Petch (1926). This form resembles conidial stage A more closely than *Papulaspora*. Globose bodies arise from hyphae which grow out from the insect on the leaf; each one bears three to six setae and consists of a mass of globose or oval cells which arise in chains from the inflated apex of the stalk. However, there is still no suggestion of true conidia. Since it is contrary to the Rules of Botanical

Nomenclature to give a separate name to the conidial stage of a fungus when the perfect form is present, this stage must be described as *Cordyceps aphodii, status conidialis A.*

The second conidial stage has all the characters of the genus *Hymenostilbe* Petch, but the same ruling must apply, so that this stage must be described as *Cordyceps aphodii, status conidialis B.*

C. aphodii therefore has two conidial stages. This is a comparatively rare condition amongst species of *Cordyceps* and has only been recorded for *C. entomorrhiza.* For this species both *Hirsutella eleutheratorum* (Nees) Petch, and *Stilbella setiformis* (Vahl) Petch (Petch, 1933) have been described as conidial stages, the former arising from the same sclerotium and the latter as lateral branches of the stroma, but in neither case has proof of the relationship been obtained in culture.

Further work must be done to determine the conditions controlling infection of the host and the spread of the disease in the field before any conclusions can be drawn on the possible value of the fungus as a means of biological control of its host.

SUMMARY

1. The larva of the pasture cockchafer, *Aphodius howitti*, is the host for a new species of *Cordyceps*, *C. aphodii.*

2. The morphology of the fungus has been described in detail.

3. There are two conidial stages, and one of these, stage A, is undescribed; the other belongs to the genus *Hymenostilbe* Petch.

4. The fungus develops in the haemocoele of the host in the form of hyphal bodies which, after death takes place, form a mycelium of hyphae and gemmae.

5. The spores of all stages have been germinated and grown on artificial media. The same type of colony is produced by all types of spore, and all colonies develop conidial stage A only.

6. The *Cordyceps* is parasitized by two other species of fungi: (*a*) a member of the Discellaceae, and (*b*) *Tilachlidium brachiatum* (Batsch.) Petch. These have also been grown on artificial media and the colonies are unlike those of the *Cordyceps.*

7. Further work is necessary before the value of the fungus as a means of biological control of the host can be assessed.

Cordyceps aphodii

Mycelium in the endosclerotium white, consisting of hyphae about 2·0 μ wide, and oval gemmae 7·5–15·0 μ long and 6·25–8·0 μ wide, which are solitary or several in a row, and terminal, intercalary or lateral on the hyphae.

Stromata caespitose, arising most frequently from the head of the sclerotium, 1·0–1·4 cm. long and 1·0–1·5 μ wide, ochraceous brown.

Stem cylindrical, equal, glabrous, composed centrally of longitudinally running hyphae, and externally of hyphae arranged irregularly at right angles to the surface. Fertile part terminal but occasionally the tip of the stroma remains sterile; distinct from the stem, ovoid to cylindrical, but often distorted due to the unequal development of perithecia; 3–4 mm.

long and about 1·5 mm. broad, and the same colour as the stalk. The surface is slightly papillate due to the dark red-brown ostioles of the perithecia. Perithecia immersed in a loosely compacted layer between the central columella of longitudinally running hyphae and the outer compact layer. They are arranged obliquely to the main axis with upwardly curved necks, about 357 μ long and 110 μ broad. The wall is several cells thick and dark brown, 6·75 μ wide. Asci 200–225 μ long and 3·8–5·0 μ broad. Cap 8·0 μ broad and 5·0 μ long. Ascospores 90–151 μ long, articles oval, flattened at each end, 5·5–7·5 μ long and 1·25–2·0 μ broad.

Status conidialis A

Develops on the stromata and from the sclerote. Conidiophores on the ends of monopodially branched hyphae, consisting of an outer layer of buff-coloured tuberculate sterile hyphae which have short lateral branches on the convex side. They enclose a cavity filled with colourless conidia, which are budded off from a group of phialides at the apex of the stalk. Conidia oval with blunt ends, 5·0–7·5 μ long and 2·0–2·5 μ broad. Conidiophores about 50–60 μ in diameter. Sterile hyphae about 3·75 μ wide.

Status conidialis B

Develops on the sides of the stromata and consists of a palisade layer of phialides and conidia. Phialides are simple, smooth, slightly broader toward the apex, terminating in a fine sterigma, 13·0 μ long and 2–3 μ broad at their greatest width. Conidia slender, pip-shaped, hyaline smooth, 5·0 μ long and 1·25 μ wide. Spores dry.

Cordyceps aphodii n.sp.

Mycelio in endosclerotio, albo, hyphis circiter, 2·0 μ latis et gemmis ovalibus 7·5–15·0 μ longis 6·25–8·0 μ latis composito; gemmis solitariis vel compluribus seriatim dispositis et terminaliter vel intercalariter vel lateraliter in hyphis.

Stromatibus caespitosis e capite sclerotii plerumque prorumpentibus, 1·0–1·4 cm. longis, 1·0–1·5 μ latis ochraceo-brunneis.

Stipite cylindraceo, aequali, glabro, intus e hyphis composito longitudinaliter dispositis, extus e hyphis irregulariter et perpendiculariter in superficie sitis.

Parte fertili terminali sed stromatis apice nonnunquam sterili, stipite distincta, ex ovoidea cylindracea, sed saepe distorta ob crescendiam inaequalem peritheciorum; 3–4 mm. longa circiter 1·5 mm. lata, eodem colore quo stipes. Superficie subpapillata ob ostiola rufo-brunnea peritheciorium.

Peritheciis immersis in strato laxe, compacto inter columellam centralem hypharum longitudinaliter dispositarum et stratum externale compactum; oblique ad axem principalem dispositis, collis sursum curvulis et circiter 367 μ longis 110 μ latis. Variete compluribus cellubis crasso, atro-brunneo, 6·75 μ lato. Ascis 200–225 μ longis 3·8–5·0 μ latis. Capite 8·0 μ lato 5·0 μ longo. Ascoporis 90–151 μ longis, articulis utrinque obtusis, 5·5–7·5 μ longis 1·25–2·0 μ latis.

Status conidialis A

In stromata et ex sclerotio oritur. Conidiophoris in apicibus hypharum monopodialiter furcatarum, strato externali hypharum tuberculatarum sterilium ochracearum compositis, quarum in latere convexo sunt breves ramuli laterales et quae cavositatem cingunt conidiis colore carentibus repletam, conidia velut gemmae gignuntun ex phialidibus in apice stipitis aggregatis. Conidiis ovalibus, apicibus obtusis 5·0–7·5 μ longis 2·0–2·5 μ latis. Conidiophoris circiter 50–60 μ diameter. Hyphis sterilibus circiter 3·75 μ latis.

Status conidialis B

In lateribus stromatum oritur, strato palisadeo phialisium et conidiorum compositus. Phialidibus simplicibus, teretibus, paullo latioribus ad apicem, sterigmate tenui terminatis, 13·0 μ longis 2·3 μ latis in parte latissima. Conidiis tenuibus ampullacea hyalinis, teretibus, 5·0 μ longis 1·25 μ latis, sporis siccis.

I would like to express my thanks to Professor J. S. Turner, in whose department this work was done, and to Associate-Professor E. McLennan, who has directed the investigation, for their continued help and advice.

I am indebted to Mr T. Petch for examining specimens of *Cordyceps aphodii* and confirming my diagnosis that it is a new species, and for naming the parasite *Tilachlidium brachiatum* (Batsch.) Petch. I would like to express my thanks for his kindness and advice.

Thanks are also due to Mr Twentyman of the Victorian Department of Agriculture, for supplying infected material, and for information regarding the habits of the larvae in the field.

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EXPLANATION OF PLATES

Plate XII, *Cordyceps*

Fig. 1. Young stromata developing from the head of the larva. × 3.

Fig. 2. Stromata at a later stage, with conidial stage A, both on the stromata and on the surface of the sclerotium. × 2.

Fig. 3. Stromata developing from the body of a sterilized larva artificially inoculated with *Cordyceps aphodii*. × 1½.

Fig. 4. Stroma covered with conidiophores of conidial stage A. × 10.

Fig. 5. Layer of conidiophores of conidial stage A fallen from the stroma in a mass. × 7.

Fig. 6. Fertile clubs of *C. aphodii* projecting above the surface of the ground. × 3.

Fig. 7. Fertile clubs developed from sclerotes incubated in the laboratory. × 3.

Plate XIII

Fig. 1. Transverse section through fertile head of *Cordyceps aphodii*. × 60.

Fig. 2. Section through an infected larva of *A. howitti*, showing hyphal bodies clustered round the tracheal tubes. × 200.

Fig. 3. Section through an infected larva of *A. howitti*, showing hyphal bodies round the outer part of the gut wall, in the fat body, and round the muscles. The black spots in the fat body are protein granules which are heavily stained with haematoxylin. × 170.

Fig. 4. Longitudinal section through fertile head of *Cordyceps aphodii*. × 20.

Plate XIV

Fig. 1. Discellaceous form on *Cordyceps aphodii*—stipitate form. The yellow waxy areas are restricted to the tips of the clavae and to two patches near the base. × 3·5.

Fig. 2. Discellaceous form on *C. aphodii*—open cup-shaped fruiting bodies on *Cordyceps* clava. × 3·5.

Fig. 3. Discellaceous form—4 single spore cultures on potato-dextrose agar three weeks old. × ⅞.

Fig. 4. *Tilachlidium brachiatum* (Batsch.) Petch on *Cordyceps aphodii* sclerotium. × 5.

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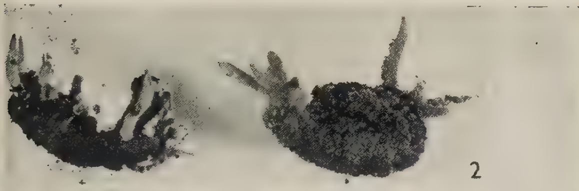

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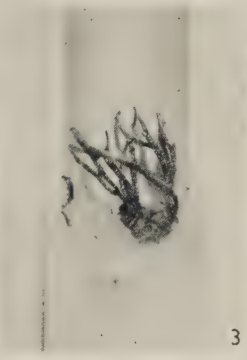

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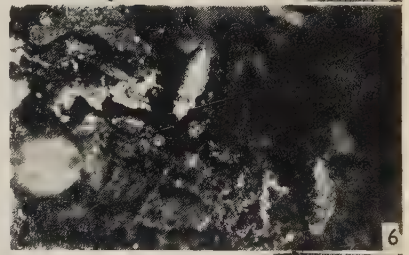

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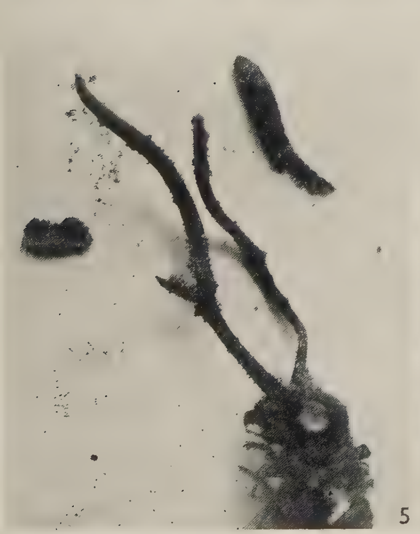

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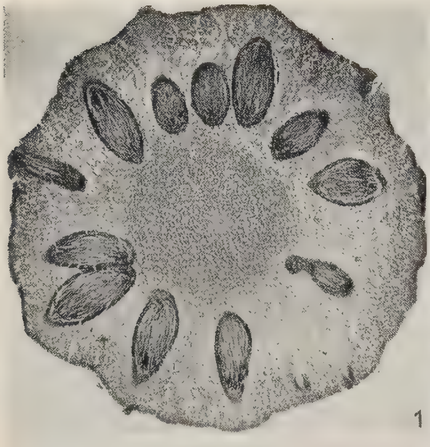
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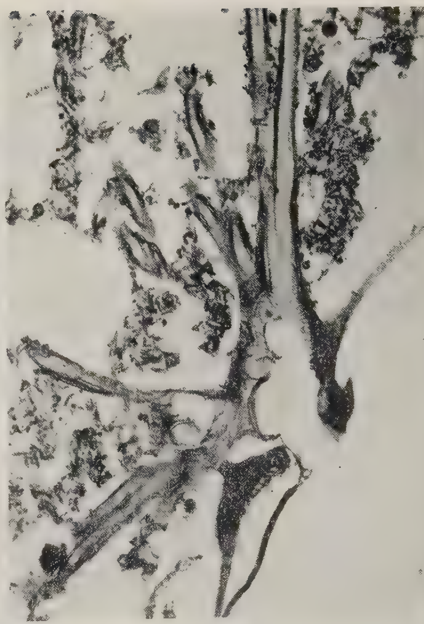
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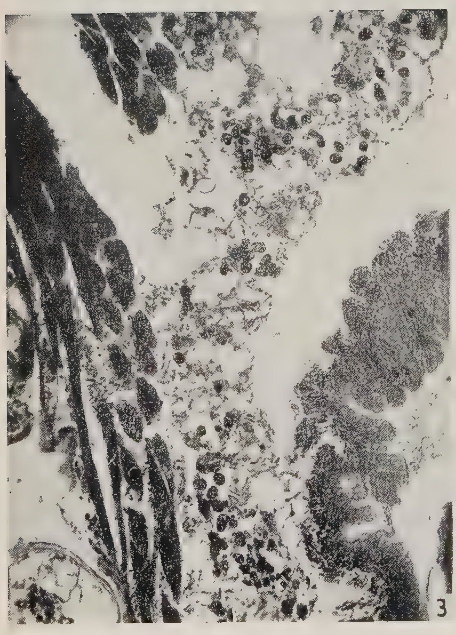
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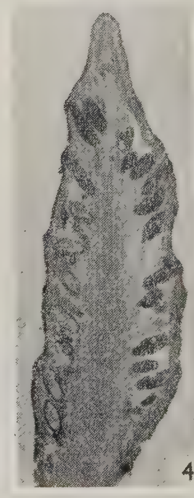
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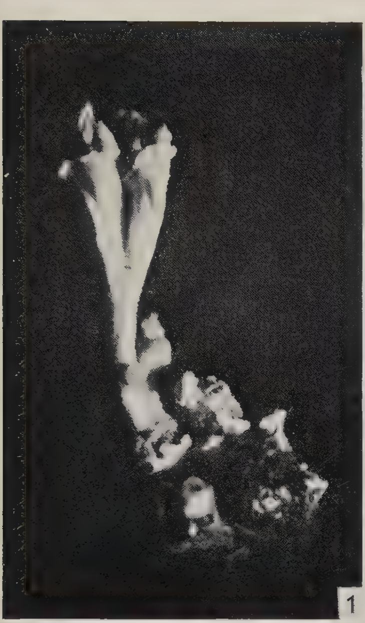

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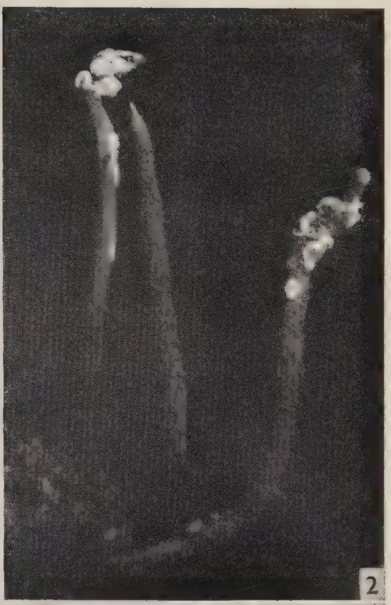

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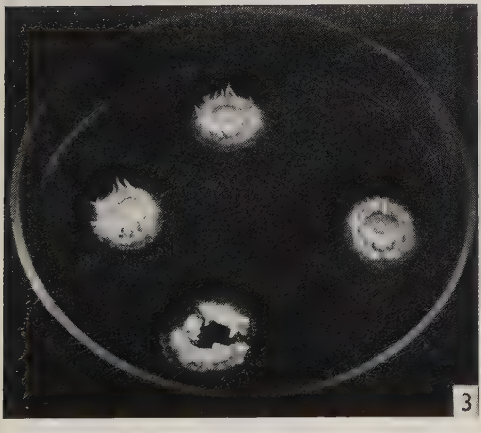

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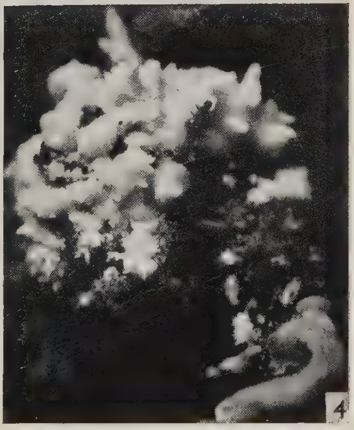


4

A COMPARATIVE STUDY OF PATHOGENIC ISOLATES OF *VERTICILLIUM*

By IVOR ISAAC

Botany School, University of Cambridge, and East Malling Research Station

(With Plates XV and XVI and 7 Text-figures)

INTRODUCTION

Reinke and Berthold (1879), in their description of the causal organism of a wilt disease of potato, stated that the diseased tissue became black or dark brown due to a blackening of the hyphae of the pathogen, which had also become septate. The short 'cells' so formed increased considerably in breadth and became spheroid giving a torulose appearance to the mycelium. When neighbouring threads become contiguous, black cell masses of varying sizes and shapes and showing no longitudinal walls, appeared in the diseased tissue. These brown 'cells' and cellular masses constituted the resting mycelium for the over-wintering of the fungus and were called by the writers 'Dauermycelien' or 'Sklerotien'. They named the fungus *Verticillium albo-atrum*.

Klebahn (1913) isolated a form of *Verticillium* from dahlia which he considered differed sufficiently from the organism described by Reinke and Berthold to entitle it to distinct specific rank, and he named it *V. dahliae*. The principal point of difference, according to Klebahn, was that his isolate formed sclerotia which arose from irregular multilateral septation and budding of the 'cells' of a single hypha and not from the anastomosis of several hyphae.

Since 1913 the relationship between *V. albo-atrum* Reinke & Berthold, and *V. dahliae* Kleb., has been the subject of much discussion. Pethybridge (1919) isolated from the skin of a potato tuber a saprophytic species of *Verticillium* producing spherical, oval or pear-shaped chlamydospores in culture. He named this *V. nigrescens* and there appears to be no other mention of this fungus in the literature. In the course of my researches on *Verticillium* Wilt of sainfoin (Isaac, 1946) and hops (Isaac & Keyworth, 1948) I made a comparative study of a large range of isolates from these and other hosts and sources, and I propose to give evidence derived from this study that three distinct and constant fungus identities can be distinguished on morphological, physiological and phytopathogenic criteria.

A list of isolates used in the experiments to be described is given in Table 1. Throughout the text the microsclerotia, dark mycelium (resting mycelium or 'dauermycelien') and chlamydospore-forming types will be referred to as types M, D and C respectively.

Table 1. *Isolates used*

Designation of isolate	Host	Source of parent culture
M 1, M 1 var.	Sainfoin	Plant Breeding Institute, Cambridge
M 2	Antirrhinum	Botany School, Field Station, Cambridge
M 3	Chrysanthemum	Cheshunt Research Station
M 4	Tomato	Cheshunt Research Station
M 5	Potato	Botany School, Field Station, Cambridge
M 6	Sainfoin	Botany School, Field Station, Cambridge
M 7	Hop	East Malling Research Station
M 8	Raspberry	East Malling Research Station
M soil 1	Soil	Soil of Plant Breeding Institute, Cambridge
D 1 var.	Cucumber	Cheshunt Research Station
D 2 var.	Tomato	Cheshunt Research Station
D 3, D 3 var.	Cucumber	Rothamsted Experiment Station, Harpenden
D 4	Hop	Hop garden, Kent
D soil 1, D 4 var.	Soil	Soil of a Hop garden, Kent
C soil 1	Soil	Soil of Plant Breeding Institute, Cambridge
C soil 2	Soil	Soil of a Hop garden, Kent

M = microsclerotial type, D = dark mycelium type, C = chlamydospore type, Soil = isolation from soil, var. = hyaline variant arising in artificial culture.

MORPHOLOGY OF ISOLATES

Resting mycelium

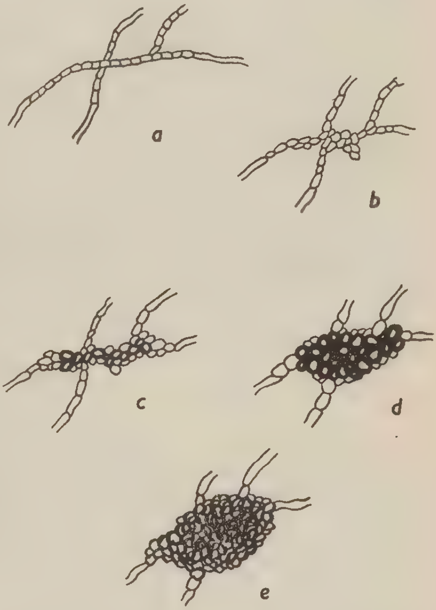
The isolates used were those listed in Table 1. In each case single-spore cultures and cultures arising from a small portion of mycelium on Dox's agar were grown in 9 cm. Petri dishes, on Dox's, Czapek's, potato-extract, potato-dextrose, Brown's, 2·5% malt-extract, 5% malt-extract, oat-extract and 2% prune-extract agar media. Observations were also made on cultures derived from single spores in hanging drops of Dox's agar medium. All the cultures were exposed to normal daylight at room temperatures. From these observations it was concluded that each isolate could be grouped into one of the following types, according to the morphology of the resting mycelium:

Type M (*microsclerotial*). The fungi in this group constantly formed microsclerotia (length approximately 50–200 μ) which gave a black appearance to the undersurface of the cultures on all media. Observations of the development of these resting bodies in hanging-drop cultures showed that after approximately five days' growth, contiguous hyphae became slightly swollen and divided by septa to form small 'cells' (Text-fig. 1 *a*). After a further six days, new 'cells' had been budded off in all planes, forming a small, almost spherical mass (Text-fig. 1 *b*). Soon, commencing from the centre of the mass, the walls of the 'cells' began to thicken and darken (Text-fig. 1 *c*), eventually forming a microsclerotium (Text-fig. 1 *d*, *e*). In some instances the 'cells' of an isolated thread multiplying by a process of budding gave rise to a microsclerotium. The best media for the development of the microsclerotial types were Dox's, Czapek's and potato-dextrose agars. In no instance were structures resembling the dark carbonized resting mycelium of type D observed in this group of cultures.

Type D (*dark mycelium*). The fungi in this group formed dark carbonized septate-resting mycelium which gave a black colour to the undersurface of the colonies. In a hanging-drop culture some hyphae were observed to become septate after eight days' growth (Text-fig. 2*a*), and within about four days the 'cells' so formed had become thickened to form black-resting mycelium (Text-fig. 2*b*). Occasionally torulose hyphae were formed by the individual 'cells' becoming swollen before the walls thickened.

No microsclerotia were observed in this group, but in Petri dish cultures on prune and potato-dextrose agars, after about three weeks' growth, black specks were seen. These were formed by the intertwining of the torulose hyphae giving rise to mycelial knots 100–500 μ in length (Pl. XV, fig. 1). In type M the resting bodies (microsclerotia) were formed from one hypha or more, but the mycelial knots of the second group were never formed from a single hypha, and while budding of 'cells' took place in the formation of microsclerotia no budding was observed in the production of mycelial knots. The latter were particularly well seen on plates where more than one colony had been grown (Pl. XV, fig. 2). It is suggested that Reinke and Berthold may have seen such structures and called them 'Sklerotien'. Berkeley, Madden and Willison (1931) and Keyworth (1942) observed these mycelial knots in their cultures of type D strains.

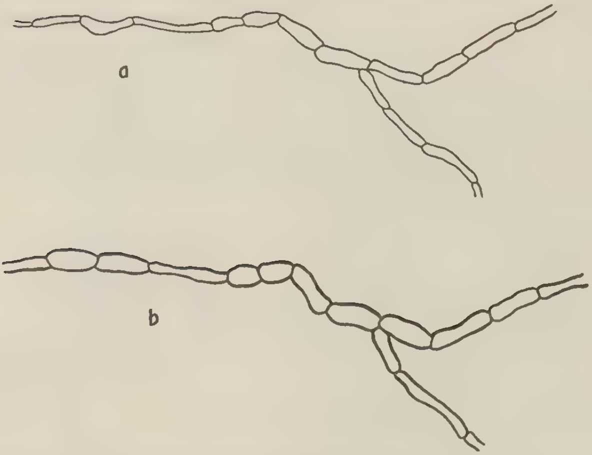
Potato-dextrose agar proved to be the best medium for the growth of this group of isolates.



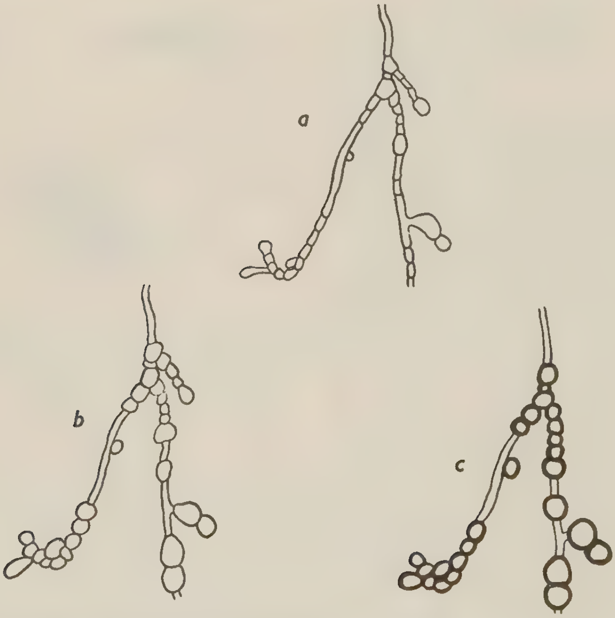
Text-fig. 1. The development of a microsclerotium of M 1 from a pure culture on a hanging drop of Dox's agar. From *a* to *e*, after five, six, eight, ten and thirteen days' growth respectively. (×375.)

Hyaline variants. Although the variants examined originated from hyaline saltants arising in Petri dish cultures of both microsclerotial and dark mycelium types, they all appeared identical in culture. Once the ability to produce black-resting bodies had been lost it was never recovered on any of the media used. The variants grew excellently on Dox's, Czapek's and potato-dextrose agar media, but conidia were produced only sparingly.

Type C (chlamydospore). The fungi in this group constantly formed spherical or pear-shaped chlamydospores (7–10 μ in diameter) giving a very dark brown to black colour to the undersurface. In a hanging-drop culture, septa appeared after about five days' growth, and these gave rise to 'cells' which within four days had become swollen and thickened to form chlamydospores (Text-fig. 3). These occurred in intercalary or terminal positions on the hyphae or were budded off the main hypha and had therefore a laterally sessile appearance. In old cultures 'cells' which did not give rise to chlamydospores became indistinct (Pl. XV, fig. 3).



Text-fig. 2. The development of dark mycelium of D 3 from a pure culture on a hanging drop of Dox's agar. *a* and *b*, after eight and twelve days' growth respectively. (×375.)



Text-fig. 3. The development of chlamydospores of C soil 1 from a pure culture on a hanging drop of Dox's agar. From *a* to *c*, after five, seven and nine days respectively. (×375.)

DIMENSIONS OF CONIDIA

Van der Meer (1925) stated that her type M differed from her type D in spore size. I found no such constant difference: the dimensions of the conidia of the type M and type D and of the hyaline variants were 3·5–10 × 2·0–4·0 μ (average 6·5 × 3·0 μ), and those of type C averaged 7·0 × 3·5 μ.

PHYSIOLOGY OF ISOLATES

In all the experiments dealing with the physiological reactions of *Verticillium* strains in culture, 9 × 2 cm. Petri dishes were generally used with the media approximately 3 mm. deep. The stock cultures from which the inocula were taken were on Dox's and potato-dextrose agars. Using a sterilized cork-borer, blocks of inoculum 3 mm. in diameter were cut and transferred to the middle of the agar plates. Usually the growths of three to five replicate plates were averaged in each experiment.

Daily measurements of two diameters at right angles to each other were recorded to the nearest half-millimetre. When the colonies did not grow out circularly, as occasionally happened, the mean of the long and short diameters was recorded. But the diameters of colonies yield an imperfect measure of the actual growth of the fungi, for as Brown (1923) stated: 'the greatest objection to the lineal expansion is that it affords in many cases no indication whatever of the amount of mycelium in the fungal colony'. However, as a general measure of the relative growth of the *Verticillium* strains, the lineal method was convenient and provided a basis for comparing the influence of one temperature on different organisms or of different temperatures on the same organism.

Temperature relations

There is considerable published work concerning the effect of temperature on the growth of *Verticillium* in culture from which it is clear that some confusion exists in the actual nomenclature adopted for the strains of fungi used. As Rudolph (1931) stated: 'Chaudhuri (1923) and Bewley (1922) have reported in detail the results of their cultural studies of *Verticillium albo-atrum* R. & B., as have Pethybridge (1916) and Van der Meer (1925). But in studying the data it is only too obvious that the first two workers were working with strains of the fungus which were not identical with that studied by the latter two.'

The aim of the present investigation was to determine and compare the temperature ranges of growth of each of the three mycelial forms of *Verticillium* described above.

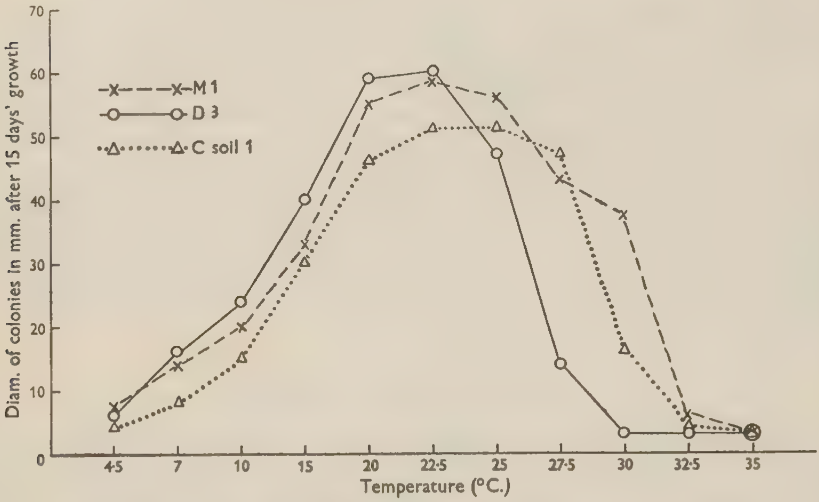
The isolates used were: (1) type M (M 1 and M 7) with a hyaline variant (M var. 1); (2) type D (D3 and D4) and their variants (D3 var. and D4 var.); and (3) types C (C soil 1).

The determinations were made on three media, viz. normal Dox's, buffered Dox's (see p. 16) and potato-dextrose agars. The range of temperatures under which the cultures were incubated was 4·5, 7·0, 10·0, 15·0, 20·0, 22·5, 25·0, 27·5, 30·0, 32·5 and 35·0° C.

Growth rates. The results for one representative of each of the three mycelial types (M 1, D3 and C soil 1) on potato-dextrose media are shown in Text-fig. 4. No appreciable deviations were recorded among the other isolates of each type and the variants closely resembled their cultures of origin in their temperature reactions. The results for each medium were also closely similar.

Cultural morphology. Wherever positive growth was recorded the morphology of the resulting mycelium was normal (i.e. true to the type of isolate employed). At 30° C., however, D3, D3 var., D4 and D4 var. budded in a yeast-like manner and no hyphal growth occurred.

Conclusions. No differences were observed in the optimum temperature for the development of the types M and D and their variants; all showed best growth at 22·5° C., while type C differed from these by growing as well at 25·0 as at 22·5° C.



Text-fig. 4. Influence of temperature upon the growth of M 1, D 3 and C soil 1 on Dox's medium.

At higher temperatures a marked difference was observed between type M, its variant and type C on the one hand, and type D and its variants on the other. The growth of the latter in contrast to the former declined very rapidly at temperatures above 25·0° C. At 30° C. the type M cultures and variant and type C form grew moderately well, whereas the types D and their variants merely produced small yeast-like colonies (Pl. XV, fig. 4). At 32·5° C. the M 1, M 1 var., M 7 and C soil 1 cultures still showed a little growth, but no development occurred in the type D forms and variants. At 35·0° C. none of the isolates showed any growth. These results conform with those of Ludbrook (1933), who stated that his *V. albo-atrum* (type D) showed no growth at 30·0° C., while all his cultures of *V. dahliae* (type M) at this temperature showed some degree of development.

The minimum temperature necessary for the growth of any of the strains appeared to be just below 4·5° C.

pH OF MEDIA

Growth effect of isolates on pH. To compare and contrast the effects of a type M (M 1), a type D (D 3) and a type C (C soil 1) form upon the pH of media, twelve cultures of each fungus were grown on each of three media: (*a*) normal Dox's agar, (*b*) Dox's agar with 0·2% ammonium nitrate substituted for 0·2% sodium nitrate and (*c*) potato-extract agar. The cultures were incubated at 22·5° C. After six hours the pH of one plate of each fungus upon each medium was tested by the 'drop' method—using the B.D.H. indicators and comparing the results with the standards in Clark's Table (1923). After twelve hours and then on each subsequent day other plates were similarly examined.

This experiment brought out no differences between the three types, all of which rapidly produced a high degree of alkalinity in normal Dox's and potato-extract media, while with the ammonium medium they rapidly increased the degree of acidity. In every case a change in the pH value was noted before any sign of growth was observed and each fungus had an apparent effect upon the medium at a considerable distance from the colony.

By the end of three days the medium in every dish of Dox's and potato-extract agars was affected throughout its bulk, although the growth of the colony had been very slight. With modified Dox's agar plus ammonium nitrate all the medium was affected after a lapse of four days, although little development of the colony had occurred.

The maximum pH value induced by three types of *Verticillium* on Dox's or potato-extract agars was the same, viz. 8·4. On modified Dox's agar the lowest pH value reached was approximately 3·2 and at this degree of acidity growth was inhibited.

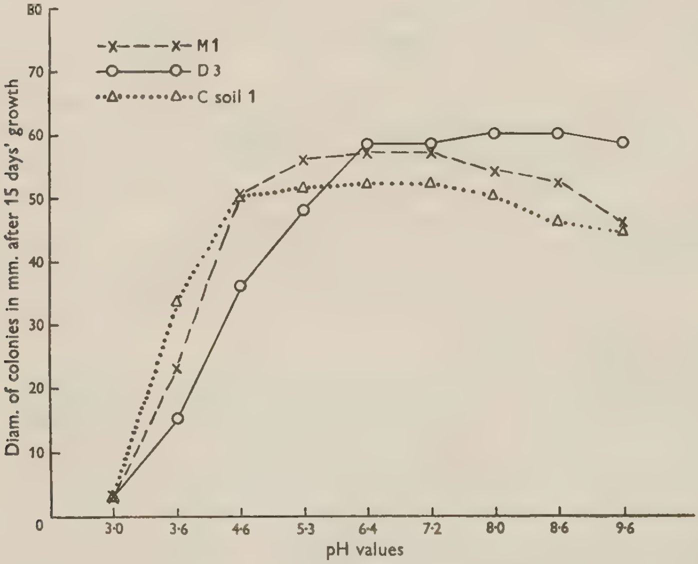
Buffering of media. Owing to the changes in the pH of the media used in the last experiment attempts were made to obtain a medium which would remain at a constant hydrogen-ion concentration throughout the growth of the fungi. Tests showed that by buffering Dox's medium with sodium and potassium phosphates a pH of 6·4 could be maintained during the growth of each type of isolate. This buffered medium is hereafter referred to as Buffered Dox's. It was also found that with potato-dextrose agar a pH of 6·4 was maintained for all isolates.

Effect of varied initial pH *on growth of isolates.* The same representatives of the three types of isolates as in the previous experiment (M1, M7, M1 var., D3, D3 var. D4, D4 var. and C soil 1) were used on the same three media (Dox's, Buffered Dox's and potato-dextrose agars). The media were adjusted to the following range of pH values: 3·0, 3·6, 4·6, 5·3, 6·4, 7·2, 8·0, 8·6 and 9·6. For this purpose normal sodium hydroxide and normal hydrochloric acid were used for the Dox's medium and 2N sodium hydroxide and 2N hydrochloric acid for the two buffered media. Three plates of each medium at each pH were poured for each isolate and all were incubated at 22·5° C.

For the same reasons as given for the graph of temperature relationships the graph shown in Text-fig. 5 shows only the results for one micro-sclerotial (M1), one dark mycelium (D3) and one chlamydospore (C soil 1) type on potato-dextrose medium.

Where an old culture of M1 was used the number of microsclerotia formed varied directly with the pH of the media: at 3·6 no sclerotia were produced, whereas at 8·0, 8·6 and 9·6 dense masses of these resting bodies appeared. Young cultures of M1 produced sclerotia abundantly on each plate where growth occurred. All the other fungi appeared normal at each pH where development took place.

This experiment brought out a marked difference between the type M, the variant M1 var., and the type C isolates, all of which showed best growth on all media between 5·3 and 7·2 and the type D isolates whose optimum pH for growth lay between 8·0 and 8·6. These results may be correlated to some extent with those of Verona and Ceccarelli (1935), whose figure 8·5, as the optimum pH for *V. albo-atrum*, corresponds very closely with my results for the type D forms, but 4·9 for *V. dahliae* is too low for the best growth of the type M isolates M1 and M7.



Text-fig. 5. Influence of pH of potato dextrose on the growth of M 1, D 3 and C soil 1.

The minimum pH value necessary for the growth of all the strains of *Verticillium* lay between 3·0 and 3·6, although at the latter value fairly good growth occurred. This does not agree with Haenseler's (1928) statement that *Verticillium* from egg-plant showed little or no growth below 4·0, or with Richardson's (1933) conclusion that *V. dahliae* grew at 2·3.

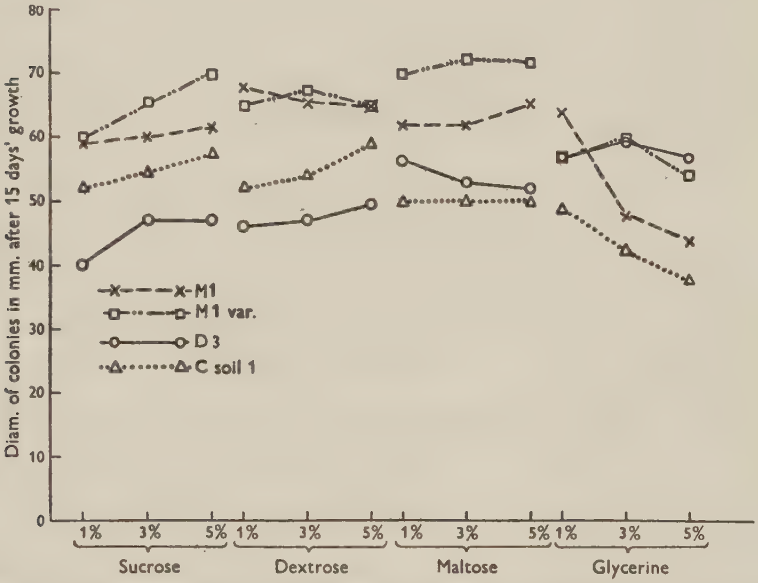
No differences were noted between the isolates in their power to modify the pH of media. As all the strains of *Verticillium* showed good development at the highest pH value used, it is not possible to state at what degree of alkalinity growth is inhibited.

VARIATION OF SOURCES OF CARBOHYDRATES

Representative strains of the three fungal types and their variants were grown on Dox's medium with sugar content varied by substituting the following sugars at 1, 3 and 5% for the normal 1·5% sucrose-sucrose, dextrose, maltose and glycerine—twelve variations of Dox's medium in all.

The isolates used were the same as were tested in the temperature-relations experiments (M 1, M 7, M 1 var., D 3, D 4, D 3 var., D 4 var., C soil 1) and four replicates of each of the twelve media were made for each isolate. The plates were incubated at 22·5° C.

Growth rates. As the variants of each type reacted similarly to each other but differently from the parent forms, one variant only (M 1 var.) is included in the following graphic summary (Text-fig. 6) of the results, together with one microsclerotial, one dark mycelium and one chlamydospore form.



Text-fig. 6. Influence of various carbohydrates upon the growth of M 1, D 3 and C soil 1.

Cultural morphology. Except for M 7 which induced a reddish brown colour in the glycerine media all the isolates on all media grew true to type.

Conclusions. Types M and D showed differences in their reactions to the various sources of carbohydrate. The former grew well on media containing sucrose, dextrose and maltose, and poorly on glycerine, while the latter developed poorly on sucrose and dextrose media and showed maximum growth on glycerine. All the variants reacted similarly to each other; D 3 var. and D 4 var. in contrast to their parent types and, like M 1 var., grew well on sucrose and dextrose while maximum growth for the three was on maltose. Growth was fairly good on glycerine.

The chlamydospore form, as also the microsclerotial isolates, showed good growth on sucrose and dextrose media and poor development on glycerine. Growth was fairly good on maltose.

No general conclusions could be drawn from the variations of sugar concentration because the effect upon the growth of the fungi was not sufficiently consistent.

VARIATION OF SOURCES OF NITROGEN

Representative strains of the three types of isolates and their variants were grown on Dox's medium containing maltose, which had proved suitable for each strain, instead of sucrose, and with the nitrogen content varied by substituting the following at 0·1, 0·5 and 1·0% for the normal 0·2% sodium nitrate—asparagine, peptone, sodium nitrate, ammonium sulphate and ammonium nitrate. It has been shown that unbuffered ammonium medium rapidly becomes too acid for the growth of *Verticillium*, so to counteract the fall in pH value a 1·0% ammonium sulphate medium was buffered with calcium carbonate (0·1, 0·5 and 1·0%) and as a comparison a 1·0% sodium nitrate medium was similarly buffered.

The isolates were the same as those used in the previous experiments and four plates were poured of each of the twenty-one media. All were incubated at 22·5° C.

Growth rates. In the graph (Text-fig. 7) showing the results, one type M, one type D, one variant and the type C isolate are included.

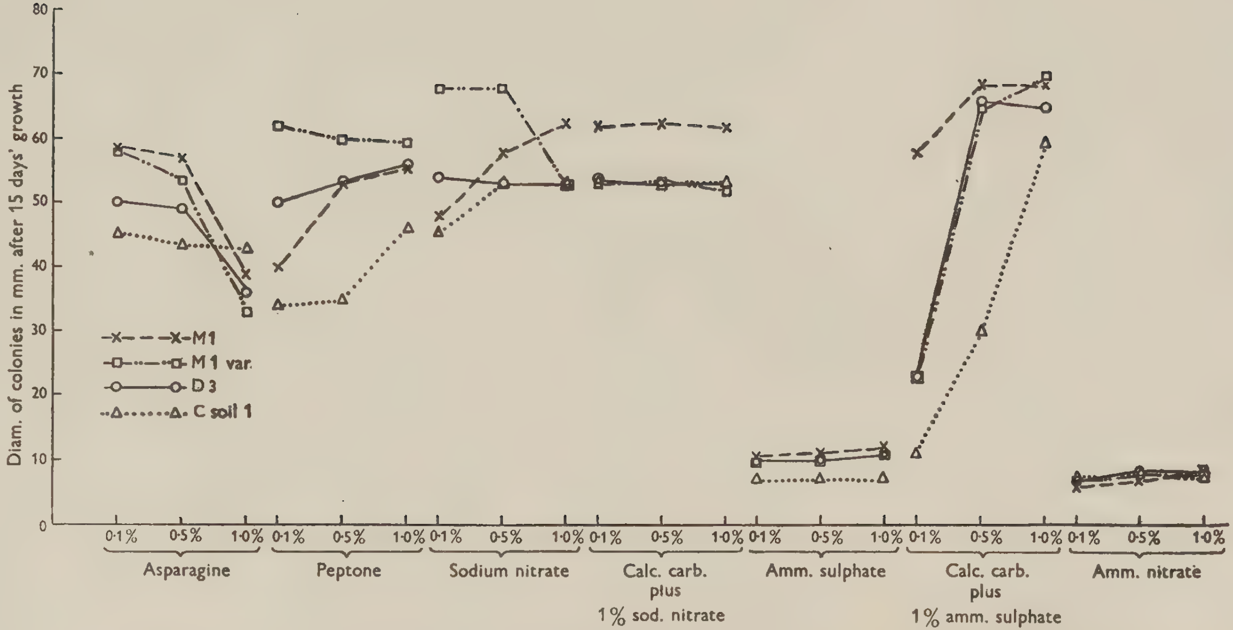
Cultural morphology. Asparagine. The type M and type C isolates appeared true to type on all asparagine media. The type D isolates, however, produced no black-resting mycelium on 1·0% asparagine, very little on 0·1%, but formed it abundantly on 0·5%. Except on 1·0%, where a small thick mat with a fawn coloured undersurface was formed, all the variants appeared normal.

Peptone. The M and C isolates grew true to type on all peptone media. The D isolate did not produce black-resting mycelium on 0·1%, very little on 0·5%, but formed it in abundance on 1·0%. Except on 0·1%, where a very thin mat with no aerial mycelium was formed, all the variants grew normally.

Sodium nitrate. All the isolates grew true to type on all the sodium nitrate media although the greatest tendency to form hyaline variants in the M and D types occurred here. The addition of calcium carbonate had no apparent effect on growth.

Ammonium sulphate and ammonium nitrate. On the unbuffered ammonium media of initial pH 5·3 all the colonies appeared as small abnormal, yeast-like clumps. No sporing occurred. Where 0·1% calcium carbonate had been added to the ammonium media the types M produced very thin white mats of irregular outline, with very few microsclerotia and no spores. The D isolates produced small thick mats with no black-resting mycelium and very few conidiophores. The variants produced small abnormal mats of mycelium with no spores. The chlamydospore form (C soil 1) isolate grew true to type except that little sporing occurred. Where 0·5 and 1·0% calcium carbonate had been added to the ammonium sulphate medium all the isolates grew true to type.

Conclusions. All the isolates were similar in showing maximum growth on media containing ammonium as a source of nitrogen buffered with calcium carbonate at 6·4. However, a slight difference between the types appeared on the sodium nitrate media, and increase in the sodium nitrate concentration caused an increased rate of growth of the microsclerotial and



Text-fig. 7. Influence of various sources of nitrogen upon the growth of M 1, M 1 var. D 3 and C soil 1.

chlamydospore forms but a decline of the dark mycelium type, greater in the case of the variants of both types.

On 0·1 and 0·5% asparagine the microsclerotial and dark mycelium types and their variants made good growth, but an increase to 1·0% resulted in slowing down development. On all the peptone media these types grew well while the chlamydospore type showed only fair growth on asparagine and peptone.

Although maximum growth of all the isolates occurred on buffered ammonium media where there was no stabilizing calcium carbonate, the plates rapidly reached a degree of acidity which inhibited further growth. Where ammonium nitrate was used the fall in pH value suggested that the fungi preferred the nitrogen of the ammonium ion to that of the nitrate even to the extent of creating a medium upon which it could make no further growth.

PATHOGENICITY STUDIES

Temperature Relations

Edson and Shapovalov (1920), working on Verticillium Wilt of potato, suggested that there were two strains of the fungus, one found in the northern regions of the U.S.A. and the other in the southern area. The southern strain, which formed microsclerotia in culture, showed a better adaptation to higher temperatures and grew fairly well at 30° C., while the northern strain in which microsclerotia were 'practically absent' adapted itself more readily to lower temperatures and did not grow at 30° C. Bewley (1922) stated that with tomato the months of June, July and August were unfavourable to the rapid progress of the disease and he suggested that control could be effected by raising the temperature of the tomato-house above 15° C., but he did not differentiate between his isolates and simply referred to his organism as *Verticillium albo-atrum*. Later, Williams (1946), working in the same laboratory, presented experimental evidence indicating that infection of a variety of tomato by *V. albo-atrum* was checked by warm moist conditions, and that by *V. dahliae* somewhat less so. Low temperatures were also found to favour infection of cucumbers by *V. albo-atrum*. Ludbrook (1933), differentiating between the two types, reported that *V. dahliae* (microsclerotial type) induced disease symptoms in egg-plant at soil temperatures of 12–30° C. but not at 32° C., whereas *V. albo-atrum* (dark mycelium type) caused disease at 28° C. and below but not at 30° C., the air temperature in each case being between 19 and 23°C.

To determine the relation between temperature and the pathogenicity of the various *Verticillium* isolates studied in the present work one type of M (M1) and one type of D (D3) isolates were compared on sainfoin and tomato as host plants, and the experiments were carried out in four glass-houses with a mean temperature of 21·5, 25, 27 and 29° C. respectively.

Sainfoin. Five common sainfoin plants (Hunter strain) were placed in each glasshouse: four were wound-inoculated with M1 isolate and one (control) was wounded only. After approximately three weeks, the five plants in the hottest house flagged and died, but when examined they showed no trace of *Verticillium* disease, which suggested that sainfoin normally cannot survive at high temperatures. The inoculated plants in all the other houses showed symptoms of Wilt after five to seven weeks, while the controls remained healthy. It is concluded, therefore, that the type M *Verticillium* is pathogenic to sainfoin at a temperature as high as 27° C.

Tomato. Parallel experiments were carried out with tomato plants inoculated with M 1 and D 3. Ten plants, singly in pots, were placed in each glasshouse immediately after being wound-inoculated just below ground level; four were inoculated with one type of *Verticillium*, four with the other type, while two, with wounds only, were kept as controls.

The plants inoculated with M 1 in glasshouses 1, 2 and 3 showed wilt after five to six weeks and the fungus was reisolated in each case, while those in glasshouse 4 displayed no wilt symptoms and *Verticillium* was isolated from one plant only. Wilted plants from other houses recovered when placed in glasshouse 4, but when replaced in their original houses quickly wilted and died. The controls in each house remained healthy.

Where type D was used only those plants in glasshouse 1 wilted, and when these were transferred to hotter houses some recovery occurred, but on being returned to glasshouse 1 they again wilted. Some of the plants from glasshouses 2 and 3 when placed in glasshouse 1 quickly showed symptoms of wilt, but on being returned to their original houses they recovered. The plants from glasshouse 4 showed no wilt, even when placed in glasshouse 1, which suggests that the temperature in the fourth house was too high for the inoculations to be successful. In reisolation attempts the fungus was obtained from all the inoculated plants in glasshouses 1 and 2, from two in the third house and from none in the fourth. The controls in each house remained healthy.

These results show that the relation of *Verticillium* wilt of tomato to high temperature differs according to the type of *Verticillium* used. Plants inoculated with M 1 and those with D 3 wilted when kept in a house with a mean temperature of 21·5° C., but higher temperatures than this (25, 27 and 29° C.) had an inhibiting effect upon the development of symptoms by D 3 in tomato, whilst M 1 induced wilt at 25 and 27° C. While it may be suggested that these results correspond to some extent with the reports of Edson and Shapovalov (1920) and Ludbrook (1933) of a definite difference in the reactions of various strains of *Verticillium* to higher temperatures, they do not conform with Bewley's conclusion that *Verticillium* wilt of tomato does not occur at temperatures higher than 24° C. Bewley's statement may have been based on work with a strain similar to the type D 3, because while the control measure which he suggested is quite effective with this type, plants infected by type M do not recover unless subjected to an indoor temperature exceeding 27° C.

CROSS-INOCULATIONS

Cross-inoculation experiments involving various host plants and the nineteen isolates were made to determine whether the strains in question could be classified on pathological criteria. Units of four to twelve plants of each host in pots or in the field were inoculated with pure cultures of the fungi by the following methods.

Wound-inoculation was used for each host. The fungi were inserted into wounds made in the wood of the main stem just below ground level, the inocula being cut from cultures on Dox's and potato-dextrose agar media. Recent 'reisolations' of the fungi were used to obviate any failure to cause infection due to loss of pathogenicity by prolonged artificial culture. Controls with wounds only were kept for each set of experiments.

The hypodermic syringe method was used only for hop. A water suspension of conidia was inserted through a hypodermic needle into the wood of the hop stems, about three feet above ground level.

The experiments were conducted in the summer months, the plants being kept in pots in a cold-frame unless otherwise stated. All the attempts to reisolate the fungus from the wilted plants at the end of each series of experiments were successful, and the isolate in each case proved to be morphologically identical with the inoculum.

Sainfoin (common), antirrhinum, cucumber, lucerne (Canadian Grimm), tomato (Kondine Red), egg-plant, strawberry (Royal Sovereign Malling 40), potato (Eclipse) and chrysanthemum in pots, and hop (Fuggle) and salsify growing in the field, were inoculated with strains of *Verticillium*. Although positive results were obtained with all the host plants except lucerne, no differences were observed between the symptoms and rate of progression of Wilt induced by microsclerotial and dark mycelium forms in antirrhinum, cucumber, and chrysanthemum, so these plants have been omitted from Table 2 which summarizes the results.

The *Verticillium* type D isolates induced symptoms in salsify, egg-plant, tomato, strawberry, potato and hop sooner than the M types, but both were similar in their action on other host plants used. Usually the rate of progression of Wilt caused by the type D was very much greater than that caused by the type M isolates, particularly in strawberry.

All the types M and D isolates from hop were pathogenic to sainfoin, but the D isolates from cucumber and tomato failed to induce Wilt in this host.

The type C isolates were not pathogenic to sainfoin, strawberry and cucumber; in the susceptible hosts the rate of development of Wilt caused by these organisms was less rapid than that induced by the D and M forms.

All the strains produced similar symptoms of Wilt. Usually the first symptom was a loss of colour in the lower leaves which later became pale and desiccated and then fell; higher leaves progressively became affected until the whole plant was defoliated. However, antirrhinum always, and hop, potato and tomato occasionally, showed 'true-wilt', i.e. all the leaves becoming flaccid simultaneously accompanied by a loss of turgor in the shoots.

Except where 'true-wilt' occurred the woody tissue of diseased plants was discoloured owing to the presence of gum and mycelium in the vessels and to the brown staining of the vessel walls. The presence of the fungus usually stimulated the production of tyloses in vessels, e.g. in potato, tomato, hops and cucumber.

Table 2. *Pathogenicity tests of* Verticillium *isolates*

	Sainfoin		Salsify		Egg-plant		Tomato		Strawberry		Potato		Hop	
Inoculum*	Wilted†	Incubation (weeks)‡	Wilted†	Incubation (weeks)‡	Wilted†	Incubation (weeks)‡	Wilted†	Incubation (weeks)‡	Wilted†	Incubation (weeks)‡	Wilted†	Incubation (2weeks)‡	Wilted†	Incubation (weeks)‡
M 1	12/12	3–5	4/5	4–6	5/5	5	4/4	4–5	3/4	10–12	8/10	3–5	12/12	3
M 1 var.	9/9	3–5	—	—	—	—	4/4	4–5	2/4	10–12	—	—	—	—
M 2	3/3	3–5	—	—	—	—	3/4	4–6	—	—	—	—	—	—
M 3	3/2	3–5	—	—	—	—	4/4	4–5	—	—	—	—	—	—
M 4	3/3	4	—	—	—	—	4/4	4–5	—	—	—	—	—	—
M 5	3/3	4	—	—	—	—	4/4	4–5	—	—	9/10	3–5	—	—
M 6	3/3	4	—	—	—	—	3/4	4–6	—	—	—	—	—	—
M 7	6/6	3–4	5/5	4–5	4/5	4–5	3/4	4–6	1/4	12	7/10	3–6	12/12	3
M 8	6/6	3	5/5	4–5	5/5	4	4/4	4	4/4	8–9	7/10	3–7	—	—
M soil 1	3/3	3–4	—	—	—	—	3/4	4–5	—	—	—	—	—	—
D 1 var.	0/12	—	—	—	—	—	4/4	3–4	—	—	—	—	—	—
D 2 var.	0/12	—	—	—	—	—	4/4	3–4	—	—	—	—	—	—
D 3	0/12	—	5/5	2–3	5/5	3	4/4	3–5	—	—	10/10	2–3	—	—
D 3 var.	0/12	—	—	—	—	—	4/4	3–4	—	—	—	—	—	—
D 4	12/12	3–5	5/5	2–3	5/5	2–3	4/4	3–4	7/8	4–7	10/10	2–3	12/12	2
D 4 var.	12/12	3–5	—	—	—	—	4/4	3–5	4/4	4–7	10/10	2–4	—	—
D soil 1	8/9	3–5	5/5	2–3	5/5	2–3	4/4	3–4	4/4	4–6	—	—	—	—
C soil 1	0/12	—	3/5	6	2/5	6	3/4	6–7	0/4	—	9/10	3–6	2/6	3–5
C soil 2	0/12	—	—	—	—	—	4/4	6–8	0/4	—	8/10	3–8	—	—

All controls in each series remained normal

* For list see Table 1.

† Numerator shows plants wilted, denominator, total plants inoculated.

‡ Number of weeks elapsing between inoculation and first symptoms of Wilt.

CONCLUSIONS AND DISCUSSION

The morphological differences between the three mycelial types of *Verticillium* in culture leading to their designation as microsclerotial, dark mycelium, and chlamydospore forms have been fully described in a foregoing section, and here it must be stressed that these differences have without exception remained constant throughout the present investigation, i.e. M type has never given rise to type D and vice versa. To these differences are now added those derived from physiological and pathological studies and summarized in Table 3.

Table 3. *Comparison of the three mycelial types*

	Type of resting mycelium				
	M	M var.	D	D var.	C
Optimum pH for growth in culture	5·3–7·2	5·3–7·2	8·0–8·6	8·0–8·6	5·3–7·2
Carbohydrate in culture	Best on sucrose and dextrose, very poor on glycerine	Best on maltose	Best on glycerine	Best on maltose	As type M
Growth in culture at 30° C.	Fairly good	Fairly good	Nil	Nil	Fairly good
Temperature and Wilt symptoms	Wilt up to 27° C.	—	Wilt at 21·5° C. but not at 25 and 27° C.	—	—
Pathogenicity	Less rapidly than type D on hop, potato, tomato and strawberry. All isolates pathogenic to sainfoin	As type M	See type M. Some isolates non-pathogenic to sainfoin	As type D	Less rapidly than types M and D. Non-pathogenic to sainfoin, cucumber and strawberry

If it is concluded from these data that we are dealing with three distinct organisms or groups of organisms the question arises which of them, if any, corresponds to the *V. albo-atrum* of Reinke and Berthold. I suggest that these workers were dealing with an organism identical with the type D isolates described above.

Reinke and Berthold, describing their fungus, stated that brown cellular 'Heaps', 'Zellhaufen', 'Dauermycelien', or 'Sklerotien', which never showed longitudinal walls, were formed when neighbouring hyphae came together, and maintained that these were not true tissue bodies or sclerotia. It is difficult to visualize a tissue body with only transverse walls.

Reinke and Berthold's drawing of their 'Dauermycelien' (Pl. XVI, fig. 5) suggests the resting mycelium of D3 (Pl. XVI, fig. 6) rather than the microsclerotia of M1 (Pl. XVI, fig. 7). In my type D isolates I observed, on prune and potato-dextrose agars (Pl. XV, fig. 2), black 'cell' masses or mycelial knots which were clearly seen to be formed by neighbouring hyphae becoming twisted about each other. The budding of the 'cells' of a single hypha or of a few hyphae, which is characteristic of the formation of sclerotia, has in no instance been observed in these isolates.

Klebahn (1913), Pethybridge (1916), Van der Meer (1925), Berkeley *et al.* (1931), Ludbrook (1933) and Van Beyma (1939–40) supported the contention that *V. albo-atrum* R. & B. does not produce true sclerotia. Bewley (1922), Haenseler (1928), Van der Veen (1930), Presley (1941) and McKeen (1943) considered the sclerotia and resting mycelium producing fungi (i.e. types M and D) as one and the same organism, viz. *V. albo-atrum* R. & B.

Wollenweber (1929) preferred to call *V. albo-atrum* R. & B. a sclerotial form. The fungus producing dark carbonized resting mycelium, he said, was rare and should be considered as a variety of *V. albo-atrum* R. & B., which he called *V. albo-atrum* var. *caespitosum.*

Rudolph (1931) maintained that Reinke and Berthold were obviously familiar with true sclerotia, because in their review of Hallier's work (1878) they stated that the structure drawn by Hallier (Pl. XVI, fig. 8) 'was the black-brown Dauermycelien of *Verticillium*'. It must be admitted that the structures drawn by Hallier were undoubtedly sclerotia, but they are certainly different from the 'Dauermycelien' drawn by Reinke and Berthold, Hallier's drawing definitely showing a tissue-like structure with longitudinal walls, which Reinke and Berthold stated was absent in their organism.

Rudolph also reported that the Centraal Bureau voor Schimmelcultuures at Baarn, Holland, issued to him as *V. albo-atrum* a fungus which on Czapek's agar formed microsclerotia. Berkeley *et al.* (1931), however, stated that they received three different cultures of *V. albo-atrum* from Baarn, Holland, all of which formed dark mycelium. Van Beyma (1939–40), publishing from this Institution, stated that the Centraal Bureau regarded *V. albo-atrum* as a form not producing microsclerotia.

Rudolph concluded that the fungus which produces only black carbonized hyphae and no sclerotia should be regarded merely as a variety, *V. albo-atrum* var. *tuberosum.* This latter is clearly synonymous with *V. albo-atrum* var. *caespitosum* Wollenw., and both, in my opinion, are simply the original *V. albo-atrum* R. & B.

If this conclusion is accepted another name must be assigned to the type M isolates used in this investigation and the writer suggests that these correspond very closely to *V. dahliae* Kleb. (Pl. XVI, fig. 9). Klebahn argued in favour of assigning specific rank to his fungus simply on its ability to produce microsclerotia as distinct from resting mycelium. Van der Meer (1925), Berkeley *et al.* (1931), Ludbrook (1933) and Van Beyma (1939–40) have supported his contention.

Table 3 provides ample grounds for dismissing the suggestion put forward by Bewley (1922), Haenseler (1928), McKeen (1943) and others that *V. albo-atrum* R. & B. and *V. dahliae* are one and the same organism. Whatever the rank accorded to them constant differences undoubtedly exist and we clearly have to deal with two organisms or groups of organisms of distinct identities. Are my types D and M then to be regarded as *V. albo-atrum* and a variety of *V. albo-atrum* respectively, or as distinct species? I was compelled for my research purposes tentatively to assign them different names, and in so doing was prompted not to add to present confusion and lengthy synonymy yet another name, unless compelled to do so on the well-established and generally accepted rules of fungus nomenclature.

Regarding type D ample data led to the conclusion already stated that this is the original *V. albo-atrum* R. & B. The remaining question was whether the difference between *V. albo-atrum* R. & B. and the type M entitled the latter to specific rank or merely to the rank of a variety of *V. albo-atrum*. If the former, then in my opinion it should be called *V. dahliae* Kleb., for Klebahn was the first to describe the distinctive structure of the microsclerotium. If the latter, then a new name must be given, e.g. *V. albo-atrum* var. *dahliae*.

In his examination of the criteria of fungus classification, Butler (1926) enumerated three main categories of distinction: morphological, biological, and a combination of both. Neither this author nor Cunningham (1926) favoured the distinction of species on biological criteria, but Cunningham regarded the division of species on such grounds as inevitable.

Dealing first with the morphological criteria, if by such Butler and Cunningham imply differences in the morphology of reproductive bodies, then the type M is not entitled to specific rank because no sexual reproductive bodies for *Verticillium* are yet known, and the D and M types have not been found to differ in the size of their conidia or conidiophores. But they have been found to differ consistently in the morphology of their resting bodies in culture, and I find it difficult to see why such a distinction should not rank at least as high as differences between asexual sporing structures.

It is true that morphologically indistinguishable hyaline saltants have appeared with each form in artificial culture, but even so, striking distinctions of a biological nature persisted, namely in their reactions to higher temperatures (30° C.) and in their optimum pH for growth. Thus a microsclerotial form and its variants can always be distinguished from the resting mycelium form and its variants on such cultural criteria.

An important physiological difference between types M and D lies in their respective maximum temperatures for growth (as pointed out above), and also in the maximum temperature at which Wilt is induced in their hosts: type M isolates can induce Wilt at 27° C., whereas type D isolates fail to cause wilt at 25° C.

It has been shown above that sometimes where temperature was equally favourable for the growth of either strain, different reactions of the fungi to differing host plants were observed (e.g. tomato, potato, hop and strawberry), type D inducing Wilt more rapidly than type M isolates. But whether pathogenicity should be taken into account in classifying these forms must be considered in the light of the fact that whereas all the type M isolates were pathogenic to sainfoin, of the type D isolates used only those from hop induced Wilt on this host. It is concluded that biologic forms of *V. albo-atrum* exist, but in my view this does not weaken the argument for considering the type M and D isolates as distinct species.

Whether the above differences, however, entitle type M to specific or merely to varietal rank depends on the definitions of these categories, and these are at present largely a matter of personal opinion and interpretation. Cunningham (1926), for example, has stated that 'a species is but a group of individuals separable from other groups placed in the same genus by the presence of certain characters whereby they may be recognized'. But it may be argued that this definition can be applied equally well to the term 'variety'.

At present, therefore, the ultimate criterion of any system of classification we accept must be convenience (i.e. usefulness, simplicity and practicability). As Butler (1926) stated: 'if we admit that there is no such thing in nature as a pure invariable homogeneous species...convenience is often the best guide where to draw the line between different species'.

Upon this basis it can, therefore, be decided that type M should be given specific rank since it has never hitherto been regarded as a variety of type D, and that to regard it as such now would cause needless confusion and inconvenience to plant pathologists. The separation of these forms into distinct species is both useful and practicable, and it is therefore suggested that the microsclerotial form should be regarded as *V. dahliae* Kleb.

Finally, the status of the chlamydospore-producing fungus has to be considered. Pethybridge (1919) described a saprophytic form of *Verticillium* which produced chlamydospores (Pl. XVI, fig. 10), the development, shape and size of which appear very similar to those formed by C soil 1 and C soil 2 (Pl. XV, fig. 3). Pethybridge maintained that this strain differed sufficiently from *V. albo-atrum* to warrant specific rank and he named it *V. nigrescens*. *V. nigrescens* Pethybr. was originally described as a saprophytic form only, whereas my chlamydospore strains were parasitic to potato, tomato, antirrhinum, etc., but again this is not sufficient to justify a subdivision of Pethybridge's species. For similar reasons to those given above for distinguishing types D and M as distinct species, it is suggested that type C should also be accorded specific rank, and it is proposed to ascribe to it the name given by Pethybridge, viz. *V. nigrescens*.

The above conclusions are exhibited in summary form in Table 4.

Table 4.

Reference	Proposed nomenclature	Synonyms
Type D. Resting mycelium form	*Verticillium albo-atrum* Reinke & Berthold	*Verticillium albo-atrum* var. *caespitosum* Wollenw. *Verticillium albo-atrum tuberosum* Rudolph
Type M. Microsclerotial form	*Verticillium dahliae* Kleb.	*Verticillium albo-atrum* R. & B.—Bewley, Wollenweber, Rudolph, Presley, McKeen
Type C. Chlamydospore form	*Verticillium nigrescens* Pethybr.	—

SUMMARY

The taxonomy of strains of *Verticillium*, forming microsclerotia, dark resting mycelium, and chlamydospores respectively, is discussed. In culture the optimum temperature for the growth of all the isolates was 22·5° C., but at 30° C. only the microsclerotial and chlamydospore strains developed. Optimum pH for the dark mycelium strains was 8·0–9·6, for the others 5·3–7·2. The microsclerotial and chlamydospore types grew well on sucrose, dextrose and maltose media and poorly on glycerine, while the dark mycelium strains developed well on glycerine, and poorly on sucrose and dextrose media. The best source of nitrogen for all strains was the ammonium ion.

The microsclerotial form was pathogenic to sainfoin and tomato at 25 and 27° C., but at these temperatures the dark mycelium form did not induce Wilt. At normal temperatures the dark mycelium types were generally most rapidly pathogenic, but only those from hops induced Wilt in sainfoin.

Arguments are put forward for retaining distinct specific rank for the microsclerotial, dark mycelium, and chlamydospore strains, viz. *Verticillium dahliae* Kleb., *V. albo-atrum* Reinke & Berth. and *V. nigrescens* Pethybr. respectively.

This investigation was begun during the tenure of a scholarship awarded by the Ministry of Agriculture and Fisheries and the Agricultural Research Council. I wish to express my thanks to Prof. F. T. Brooks, who suggested the investigation, for his interest and advice throughout, and to the Agricultural Research Council and East Malling Research Station for means to continue the work in conjunction with research on *Verticillium* Wilt of hops under a special grant from the former. I am also much indebted to Dr R. V. Harris for advice and criticism in the preparation of this paper. The material has been used as part of a thesis presented for the degree of Ph.D. in the University of Cambridge.

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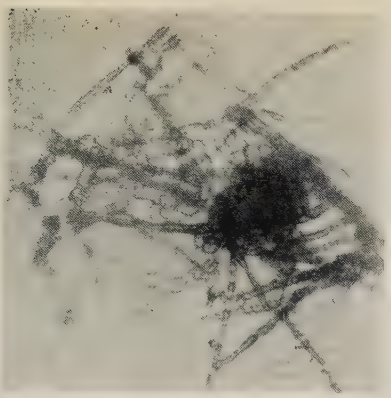

Fig. 1

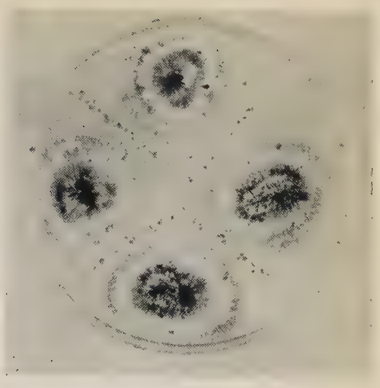

Fig. 2

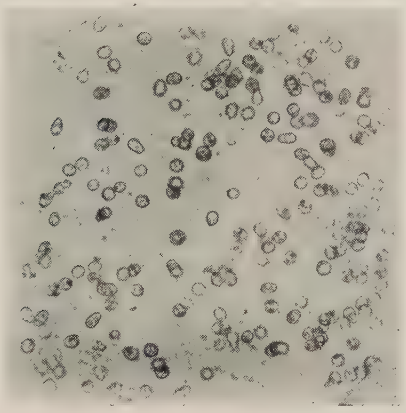

Fig. 3

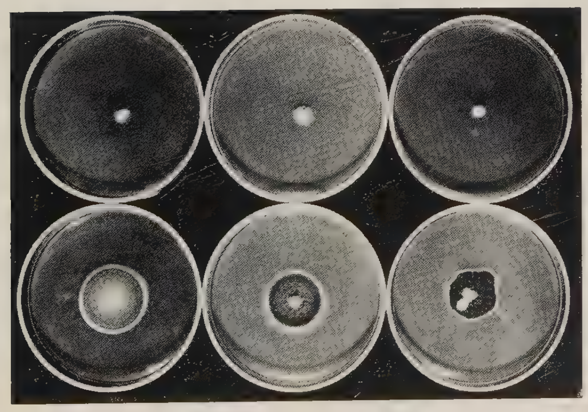

Fig. 4

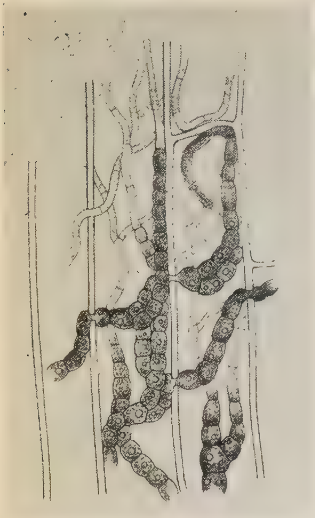

Fig. 5.

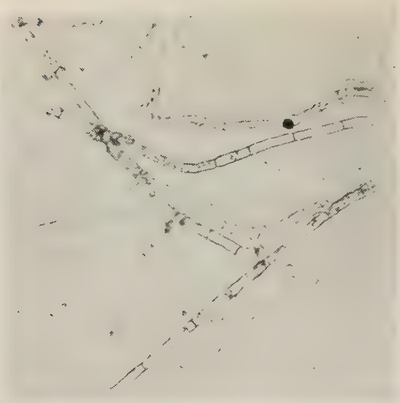

Fig. 6

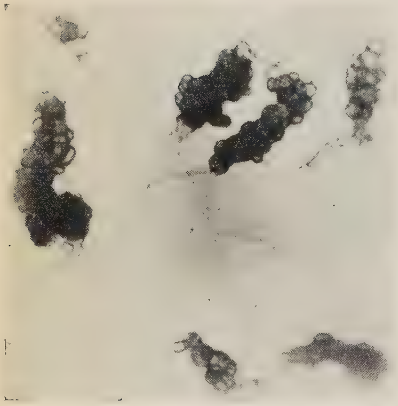

Fig. 7

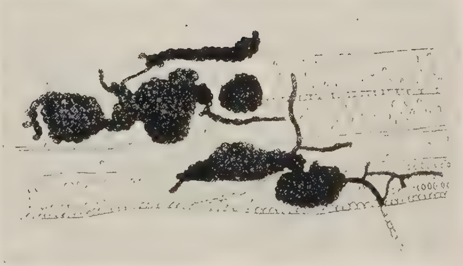

Fig. 8

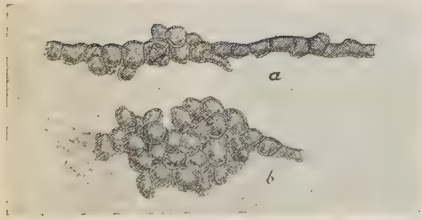

Fig. 9

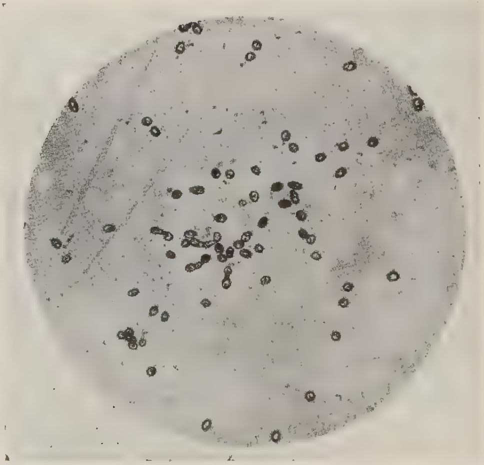

Fig. 10

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EXPLANATION OF PLATES

Plate XV

Fig. 1. Mycelial knots (due to twisting about each other of torulose hyphae) of D 3 from a pure culture on prune-extract agar. (×332.)

Fig. 2. Four pure cultures of D 3 on prune-extract agar. Note the small black resting bodies (mycelial knots) formed where the cultures meet.

Fig. 3. Chlamydospores of C soil 1 from a pure culture on Dox's agar. Note mycelium almost invisible. (×332.)

Fig. 4. D 3 (top row) and M 1 (bottom row) after fifteen days' growth at 30° C. on, left to right, potato dextrose, Dox's and Buffered Dox's media.

Plate XVI

Fig. 5. Photographic reproduction of 'Dauermycelien' or 'Sklerotien' drawn by Reinke and Berthold. (×350.)

Fig. 6. Dark mycelium of D 3 from a pure culture on Dox's agar. (×332.)

Fig. 7. Microsclerotia of M 1 from a pure culture on Dox's agar. (×332.)

Fig. 8. Photographic reproduction of sclerotia-like body drawn by Hallier.

Fig. 9. Photographic reproduction of sclerotia-like body drawn by Klebahn. (×516.)

Fig. 10. Reproduction of photograph of chlamydospores of *Verticillium nigrescens* Pethybr. (×310.)

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STACHYBOTRYS DICHROA GROVE

By G. R. BISBY and M. B. ELLIS
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(With 2 Text-figures)

In 1943 the senior author concluded that *Stachybotrys dichroa* Grove (1886) was young *S. atra* Corda. Only two collections were then known, and the spores and phialides showed much resemblance to those of the common and rather variable *S. atra*. However, recent collections have now demonstrated that *S. dichroa* is evidently not uncommon on old herbaceous stems, particularly in damp areas, and that it is clearly distinct from *S. atra*, which occurs in soil and from soil passes to damp cloth, paper, and other substrata. The following collections of *S. dichroa* have been examined:

On dead thistle stem [*Cirsium palustre*], Bradnock Hayes, near Little Sutton, 10 October 1885; ex type collection in Herb. W. B. Grove, Birmingham University (slide filed as Herb. I.M.I. 18,006).

On dead stems of *Epilobium* [*hirsutum*], Hebden Bridge, 30 June 1900, ex Herb. C. Crossland in Herb. Kew (slide filed as Herb. I.M.I. 19,217).

On dead stems of *Acanthus*, Lambourne Hill, Perranzabuloe, West Cornwall, 27 August 1944, F. Rilstone, No. 4445 (Herb. I.M.I. No. 555).

On dead stems of *Filipendula ulmaria*, Wheatfen Broad, near Surlingham, Norfolk, 9 June 1946, E. A. and M. B. Ellis (Herb. I.M.I. No. 5608*a*).

On dead umbelliferous stems, Thornton-le-Dale, Yorkshire, 12 April 1947, J. Webster, University College Hull Herb. No. 100 (Herb. I.M.I. No. 17,592).

On dead stems of *Angelica sylvestris*, grounds of University College, Exeter, 14 September 1947, J. Webster (Herb. I.M.I. No. 17,506).

Very thin 'Necol' mounts show that, in nature, *Stachybotrys dichroa* produces little or no superficial mycelium; narrow hyphae, 1–3 μ in diameter, are immersed in the substratum and only the upright hyaline conidiophores with their black shining heads of spores appear above the surface. The conidiophores arise close to one another and, as in *S. atra*, the slimy spore masses occasionally coalesce.

The phialophores are simple, septate (2–7 septa), straight or slightly curved, smooth-walled, hyaline and average 157 μ (range 86–270 μ) long; each arises from a bulbous base, 12·2 (8–20) μ in diameter, and tapers from 8·5 (5–11) μ immediately above the bulb to 4·4 (3·5–7) μ below the apex, then swells again to form a small head 6·6 (5–9) μ in diameter. The swollen apex bears a single ring of five to six smooth-walled, hyaline phialides, 11·9 (9–17) μ long and 4·4 (3·5–6) μ in diameter at their widest points; they are almost cylindrical, rounded at the apex and are often slightly narrower at the base, but never obovate or pyriform as in *S. atra*.

The conidia are produced singly and successively in slime at the apex of the phialide and form black heads of spores 38 (20–90) μ in diameter. When

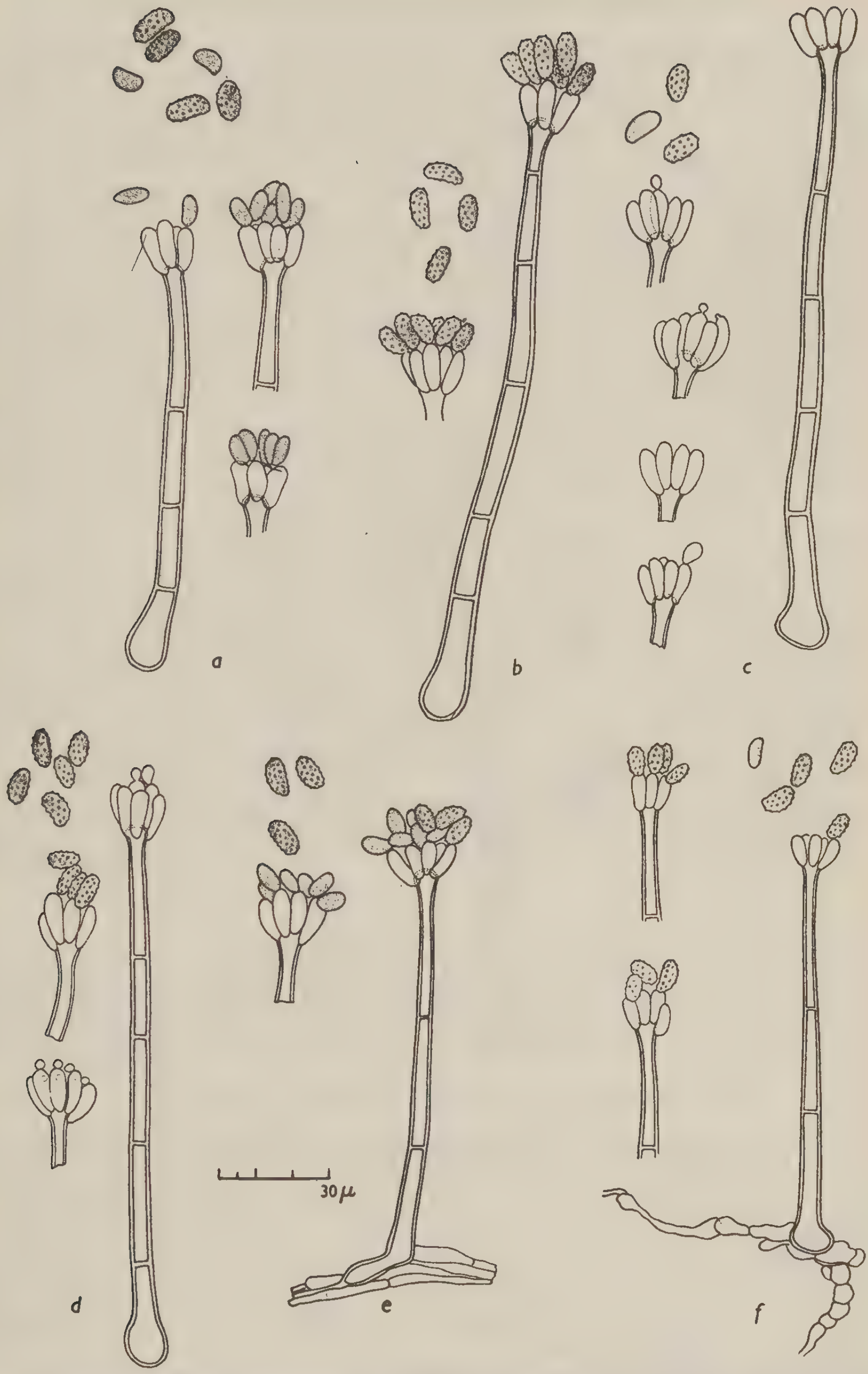

Fig. 1. *Stachybotrys dichroa*: phialophores (conidiophores), phialides, and conidia, × 500. *a*, ex type collection (I.M.I. 18,006). *b*, I.M.I. 19,217 ex Herb. Crossland. *c*, I.M.I. 5608*a* on *Filipendula*. *d*, I.M.I. 17,506 on *Angelica*. *e*, ibid., from culture after 19 days on P.D.A. *f*, I.M.I. 17,592 from culture after 19 days on P.D.A.

young the conidia are smooth-walled, pale olivaceous brown, ovoid or almost cylindrical, sometimes slightly curved, obtuse at the apex and often obliquely attenuated at the base; as they mature they become dark, finally almost black, and rough-walled. Mature conidia are 9·8 (8–12) μ long with a maximum diameter of 4·9 (4–6) μ. In fresh collections two large guttulae may be seen in the conidia, but these disappear with age.

Culturally *S. dichroa* and *S. atra* are quite distinct, colonies of the former being a bright orange colour in reverse on potato dextrose agar, in contrast to the dark olive colour usually shown by *S. atra*. Although only six collections of *S. dichroa* have so far been studied (two of them also in culture), their uniformity indicates that the species is distinctive and easily recognized. It is curious that Grove's species should have remained almost unknown for so long.

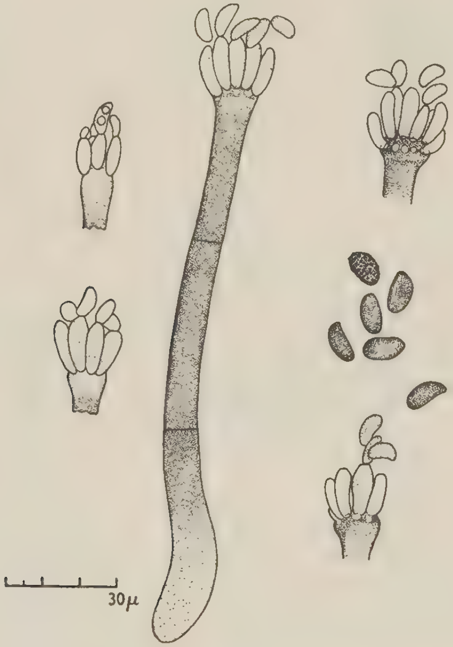

Fig. 2. *Stachybotrys* sp., I.M.I. 16,185 on *Oenanthe*, Guernsey, × 500.

There is another, apparently so far undescribed, species of *Stachybotrys* with simple phialophores, which also occurs on dead herbaceous stems. Only one collection has been made: I.M.I. No. 16,185, on dead stems of *Oenanthe crocata*, La Bouvée, Guernsey, C. Isl., 19 June 1947, E. A. and M. B. Ellis. A description and figure of this fungus are appended, and it is being held for the time being as a taxonomically distinct species, but pending the receipt and examination of further collections is not being named and described as new.

Mycelium hyaline, septate, branched, 1–3 μ in diameter, embedded in the substratum. Upright phialophores simple, 1–2 septate, straight or slightly curved, smooth-walled, smoky grey to black, 145 (120–180) μ long;

base paler and somewhat swollen but not markedly bulbous, 12·3 (11–14) μ in diameter, tapering above to 6·9 (6–9) μ, then swelling again to 10·6 (9–12) μ. The phialides are borne in one or two rings around the swollen apex; they are smooth-walled, hyaline at first, later smoky grey to black, cylindrical to obovate, often truncate at the apex, 13·7 (11–16) μ long and with a maximum diameter of 5 (4–6) μ.

The conidia are somewhat kidney-shaped, especially when young, rounded at the apex and sometimes obliquely attenuated at the base, and remain smooth for a long time; even when they had turned almost black, very few rough-walled conidia were seen; length 10·1 (9–11) μ; maximum diameter 6·2 (4·5–8) μ.

The heads of conidia do not slime down in this species to the extent that they do in *Stachybotrys dichroa* and *S. atra*; partial chains of conidia are frequent.

In cultures on potato-dextrose agar at room temperature the colonies in reverse have a pinkish orange colour, similar to that of *S. dichroa*. The rate of growth is about half that of *S. dichroa* and the 'standard' culture of *S. atra*.

A word should be added regarding *S. subsimplex* Cooke, which Bisby (1943) thought might be the same as *Memnoniella echinata* (Riv.) Galloway. Zuck (1946) later found that *M. echinata* may sometimes produce spores in slimy heads rather than the usual chains, which strengthens the view that the two so-called species may be identical. As Zuck remarks, this leaves the name *Stachybotrys subsimplex* as doubtful, unless it refers to the '*Stachybotrys* phase' of *Memnoniella echinata*. The latter proves to be a common fungus, as Bisby (1945) also reported. Incidentally, Zuck's observations throw doubt on the distinction between *Stachybotrys* and *Memnoniella* The genus *Gliobotrys*, to which von Höhnel referred *Stachybotrys dichroa*, still seems superfluous to us, though the type species, *Gliobotrys alboviridis* Höhnel, requires restudy.

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ON *APHANOMYCOPSIS BACILLARIACEARUM* SCHERFFEL, *A. DESMIDIELLA* N.SP., AND *ANCYLISTES* SPP. IN GREAT BRITAIN

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(With 6 Text-figures)

I. *APHANOMYCOPSIS BACILLARIACEARUM* SCHERFFEL

Aphanomycopsis is at present represented by a single species *A. bacillariacearum*, described by Scherffel (1925). Sparrow later described specimens from America (1933) and recorded it from England (1936).

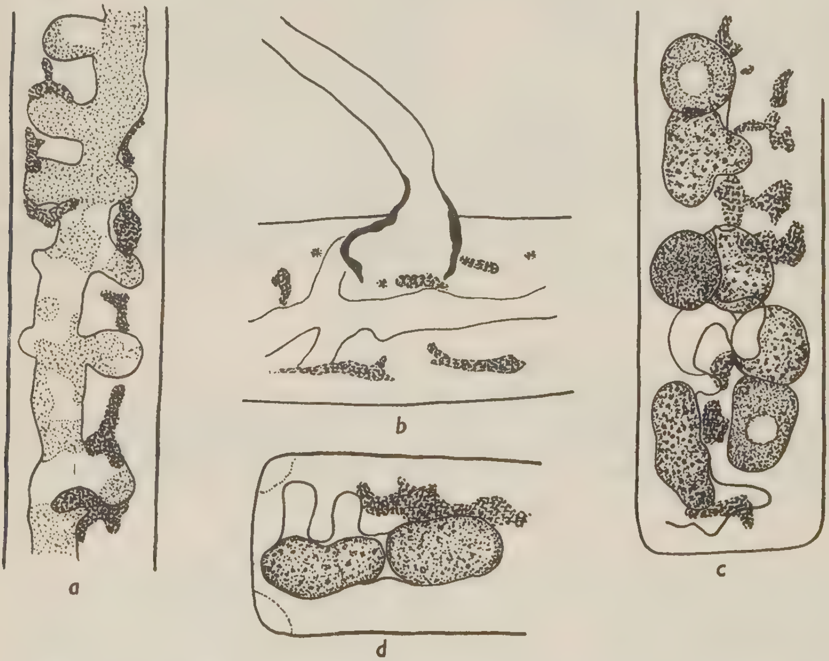

Fig. 1. *Aphanomycopsis bacillariacearum*. *a*, immature tubular thallus. *b*, empty sporangium, with exit tube which is thickened at the base, to form the so-called spreading apparatus. *c*, *d*, resting spores. All × 500.

A few specimens clearly belonging to this species were found parasitizing *Pinnularia* sp. from Blelham Bog, near Wray Castle in January 1947. The sporangium consists of a long non-septate tube 150 μ long, 13 μ in diameter, with short lateral branches (Fig. 1 *a*). The exit tube, up to

75 μ long by 9 μ in diameter, is considerably thickened and inflated at the base (Fig. 1 *b*) to form the so-called spreading apparatus concerned with separating the valves of the diatom cell. In one specimen the exit tube had bifurcated outside the host cell. The spherical primary zoospore cysts, grouped around the mouth of the exit tube, are 10–12 μ in diameter and from them emerge the secondary biflagellate swarmers. Young stages in development of the resting spores only were seen (Fig. 1 *c*, *d*). There is no sexual process and the resting spores are formed from contracted portions of the protoplasm.

II. **Aphanomycopsis desmidiella** n.sp.

A. bacillariacearum appears to be limited to diatoms as do the other freshwater representatives of the Ectrogellaceae. What is considered to be a new species of *Aphanomycopsis* was found parasitizing a desmid (*Netrium digitus* (Ehrenb.) Itzigsch & Rothe) in a Sphagnum pool at Batemanfold, Lancashire, during late September 1946. An account of its life history follows.

The encysted zoospore germinates on the surface of the host cell producing a tube which penetrates the wall and forms within, a tubular swelling (Fig. 2 *c*). This elongates, branches and finally forms a tubular non-septate contorted thallus 5–13 μ in diameter, filling the algal cell. The original zoospore case persists on the outside of the host wall and the first-formed portion of the thallus immediately within the wall often becomes swollen and may appear to be slightly thickened (Fig. 2 *h*). By these characters it is often easy to find the original place of infection and to determine the number of individual thalli present (rarely more than two) in a single cell. The fungal protoplasm contains numerous highly refractive globules surrounding a central vacuole (Fig. 2 *d*). One or two exit tubes push through the algal wall and grow into the surrounding water. They usually emerge from the ends of the *Netrium* cell (Fig. 3 *a*), but sometimes they may push through the lateral wall. The exit tube is equally cylindrical throughout its length (5 μ in diameter) and varies from 10–185 μ long (the majority are 50–100 μ long). Growth in length of the exit tube is rapid; one observed under a cover-slip over a period of two and a half hours showed the following elongation in successive half hours: 15, 23, 16, 9, 7, 6 μ.

The actual cleavage of the protoplasm into the primary zoospores and their escape was never observed, but two specimens were seen in which they had recently emerged. It is evident that they do not swarm but form a motionless mass at the mouth of the exit tube. The encysted primary zoospores are spherical, 6–7·6 μ in diameter with granular protoplasm and a few refractive globules (Fig. 2 *e*). The number of cysts formed may be up to sixty. After about an hour the naked secondary swarmer emerges (Fig. 2 *g*). A few minutes after emergence, the flagella appear and the zoospore after oscillating for some time swims away. Its movement is smooth-gliding with sudden stops and changes in direction. The zoospores are somewhat bean shaped 8 × 7 μ with two vacuoles, a small mass of refractive globules and two flagella (Fig. 2 *a*); the longer posterior flagellum is dragged behind, while the shorter more active one is anterior.

No evidence of sexuality has been found in the development of the resting spore. As Scherffel described for *Aphanomycopsis bacillariacearum* it appears to be developed from a rounded portion of the thallus protoplasm; from three to seventeen may occur lying loose in the expanded thallus wall. The spore is spherical to oval, 9–20 μ in diameter, with a thin wall and dense contents when young (Fig. 3*c*). As the spore matures, the wall thickens and becomes two-layered. The outer is highly refractive and on

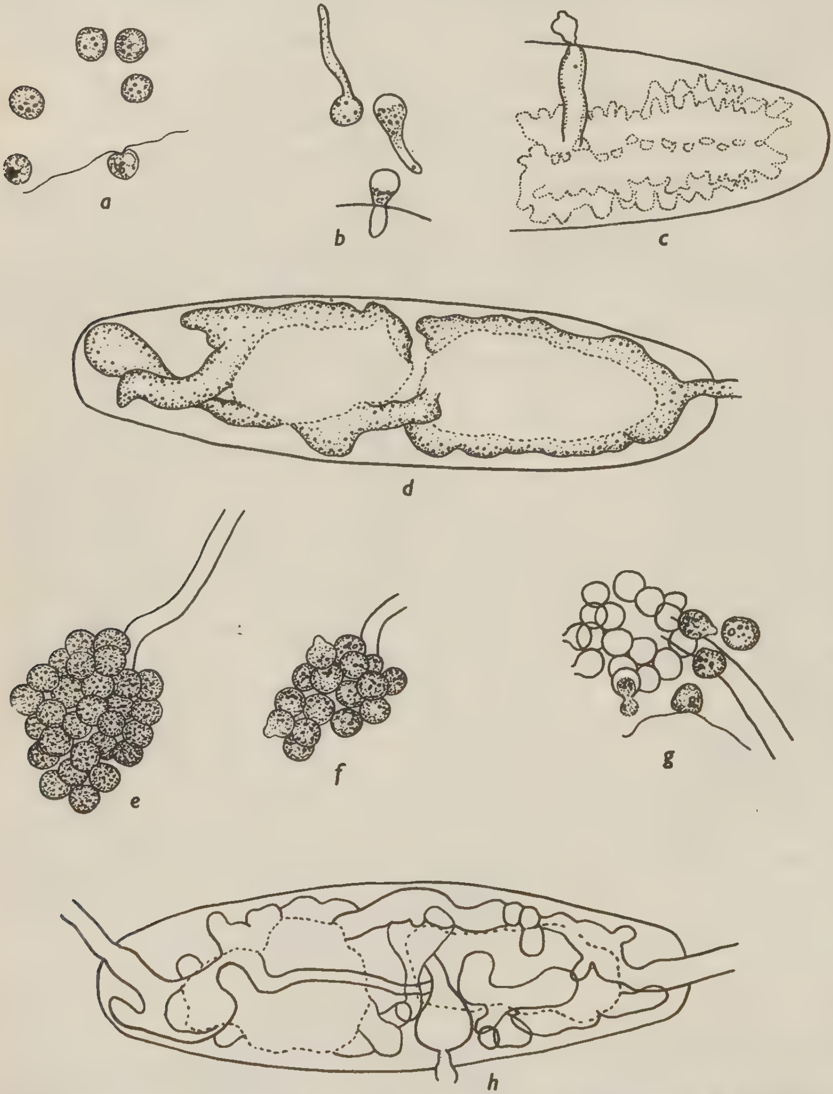

Fig. 2. *Aphanomycopsis desmidiella.* *a*, secondary zoospores. *b*, young stages in germination of zoospores; the two uppermost have germinated away from the algal cell. *c*, empty shrivelled zoospore case on algal wall, young unbranched tubular thallus inside. *d*, thallus with developing discharge tube. *e*, encysted primary zoospores. *f*, encysted primary zoospores, two with a papilla. *g*, empty primary zoospore cysts; emerging and free secondary zoospores. *h*, empty sporangium, swelling of thallus immediately beneath empty zoospore case clearly visible. *b*, *c*, *h*, × 525; *a*, *d*–*g*, × 500.

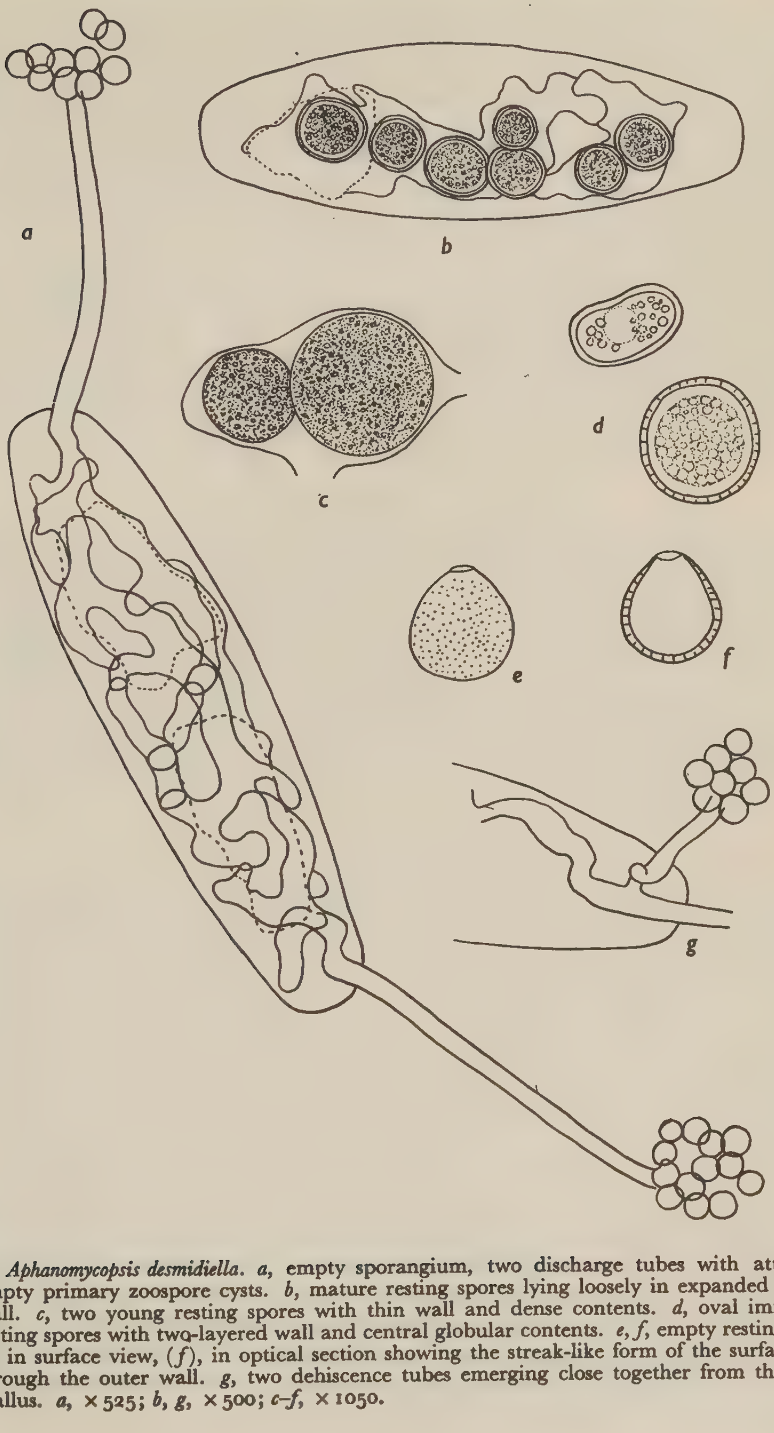

Fig. 3. *Aphanomycopsis desmidiella*. *a*, empty sporangium, two discharge tubes with attached, empty primary zoospore cysts. *b*, mature resting spores lying loosely in expanded thallus wall. *c*, two young resting spores with thin wall and dense contents. *d*, oval immature resting spores with two-layered wall and central globular contents. *e*, *f*, empty resting spore (*e*) in surface view, (*f*), in optical section showing the streak-like form of the surface dots through the outer wall. *g*, two dehiscence tubes emerging close together from the same thallus. *a*, × 525; *b*, *g*, × 500; *c*–*f*, × 1050.

a dehisced empty specimen is seen to be covered with numerous dots (Fig. 3*e*). These markings in optical section appear to penetrate the outer wall (Fig. 3*f*); whether they are pores or more highly refractive thickening bands is unknown. The inner wall is hyaline and encloses the central content which consists of numerous refractive globules (Fig. 3*d*). How the resting spore germinates remains unknown. One empty resting spore with a dehiscence pore is shown in Fig. 3*e*, *f*. After treatment with chlor-zinc-iodide the thallus, discharge tube and resting spore wall stain pink; the zoospore case gives no reaction.

Because of the striking difference in the host it does not seem advisable to suggest that the present species is identical with *A. bacillariacearum*, although morphologically it differs from that species in very minor characters, notably in the size of the secondary swarmers, in the absence of thickening in the exit tube where it penetrates the host wall and in the sculpturing of the outer membrane of the resting spore. The binomial *A. desmidiella* is proposed for this parasite.

Aphanomycopsis desmidiella n.sp.

Thallus endobiotic, branched, holocarpic, non-septate, forming a single zoosporangium. Zoosporangium 5–13 μ in diameter, dehiscing by one to two exit tubes (10–185 μ long × 5 μ in diameter). Primary zoospores (probably non-flagellate), forming ten to sixty spherical cysts (6–8 μ in diameter) at the mouth of the exit tube. Secondary zoospores (8 × 7 μ) kidney-shaped with two lateral flagella. Resting spores endobiotic, spherical to subspherical, 9–20 μ in diameter, with a thick colourless wall and containing numerous small refractive globules; germination not observed.

Parasitic in *Netrium digitus* (Ehrenb.) Itzigsch & Rothe in Batemanfold, Lancashire, England.

Aphanomycopsis desmidiella sp.nov.

Thallus endobioticus, holocarpicus, ramosus, aseptatus, zoosporangium singulum generans. Zoosporangium 5–13 μ diam., per 1–2 tubulos (10–185 μ longos 5 μ latos) dehiscens. Zoosporae primariae (verisimiliter non-flagellatae) 10–60 cystos sphaericos (6–8 μ diam.) ad ostiolum tubuli efficientes. Zoosporae secundariae (8 × 7 μ) reniformes, flagellis lateralibus binis. Sporae perdurantes endobioticae, sphaericae vel subsphaericae, 9–20 μ diam., tunica crassa hyalina, globulos refractivos numerosus parvos continentes. Germinatio non visa.

Hab. Parasiticus in *Netrio digito* (Ehrenb.) Itzigsch et Rothe, Bateman fold, Lancashire, Anglia.

Certain other incompletely described fungi found in desmids may be referable to this genus or even to *Aphanomycopsis desmidiella*. It is probable that a species of *Aphanomycopsis* caused Archer (1860) to claim that zoospores occurred in desmids. Archer noted that naked bodies emerged through tubes which had grown out from *Docidium ehrenbergii* = *Pleurotaenium ehrenbergii* (Brèb) De Bary, at the junction of the two semi-cells; these bodies encysted and again emerged as ovate or pyriform, ciliated swarmers.

West & West (1906) identify a fungus found in *Pleurotaenium ehrenbergii* with that of Archer. They, however, mention that the thallus is divided into two parts by a septum. It is possible that this septum may be an artefact, since, according to their figure, if a septum is present only the portion of the thallus in one semi-cell has dehisced, whereas that in the other semi-cell having no exit tube, should still possess its contents, which are not figured. Both these records are incomplete, and until the structure of the resting spore is known their actual identities remain uncertain.

If Wests' fungus is divided into two parts it would then resemble the form described by Tokunaga (1934) in *Surirella* sp. and *Navicula* sp., where the thallus is septate at indefinite intervals into a number of cells, each component cell functioning at maturity as a sporangium or oogonium. As pointed out by Sparrow (1943, p. 537), such septate fungi cannot be included in the Ectrogellaceae as originally defined by Scherffel (1925) and may represent the type of a new genus. Karling (1942), however, has revised Scherffel's original diagnosis of *Aphanomycopsis bacillariacearum* to include the septate form described by Tokunaga which is considered by the author to represent at least the type of a new species.

III. *ANCYLISTES* spp.

The genus *Ancylistes*, although known for a long time from the Continent (Pfitzer, 1872), has only recently been discovered in America (Berdan, 1938), and until now has not been recorded from Great Britain. On the two occasions I have found *Ancylistes* it was rare and only a few stages in the life history were observed. In view of this, I refer to them as *Ancylistes* spp. although it is possible that one may be new to science.

Since Berdan (1938) removed *Ancylistes* to the Entomophthorales, due to the discovery of aerial-borne conidia, no further support of her work has appeared in the literature. In the one specimen I found parasitizing *Closterium* sp. from the Clay Pond, Wray Castle, in September 1946, I too observed such conidia (Fig. 4).

Up to the present, species of *Ancylistes* have been recorded only in *Closterium*, whereas the second fungus, which occurred on a muddy path leading to Three Dubs Tarn, Claife Heights, near Sawrey, Lancashire, parasitized *Tetmemorus granulatus* Bréb. Although *T. brebissonii* was also present, it was not attacked. The specificity of these organisms was also noted by Berdan, for *Ancylistes closteri* and *A. pfeifferi* occurred together in the same pool but attacked different species of *Closterium*.

The fungus in *Tetmemorus* differs from other species since all the external hyphae grow to the centre of the desmid and then push out through the broken junction of the two semi-cells (Fig. 5). No conidia or resting spores were observed.

The intramatrical mycelium is unbranched, often extending to the length of the host cell, 98–115 μ long by 10–17 μ broad with rounded ends and containing regularly arranged refractive granules. More than one infection may occur in a single host cell (Fig. 6*e*, *g*, *h*). At maturity, the thallus is cut up into three to twelve segments, 9–25 μ long by 10–17 μ broad. Each segment produces one external hypha which grows rapidly, forming septa posteriorly and contains highly refractive granules collected together in certain regions (Fig. 6*h*, *i*). Branched external hyphae are commonly seen. One thallus was found which did not become cut up into segments, and produced a single broad external hypha (Fig. 6*h* (*x*)). Infection of new hosts is by the swollen end of an external hypha in contact with the wall. The appressorium is very weakly developed and in many it could not be found. Soon after the host cell is entered the infection tube disappears. Early stages in development are shown in Fig. 6*a–c*. The

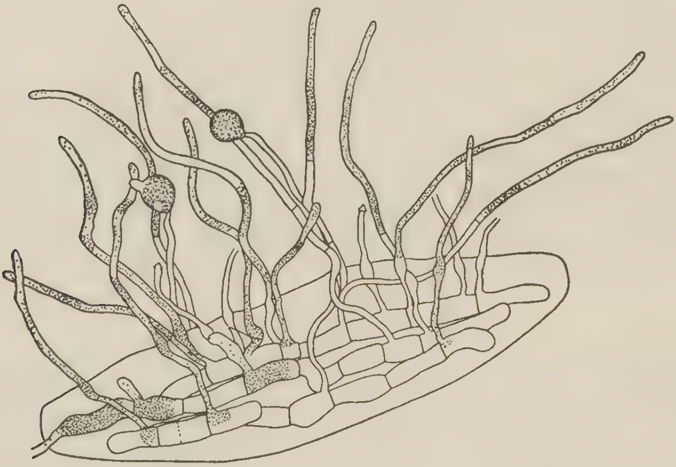

Fig. 4. *Ancylistes* sp. with external hyphae and conidia in *Closterium* sp. from the Clay Pond, Wray Castle. × 256.

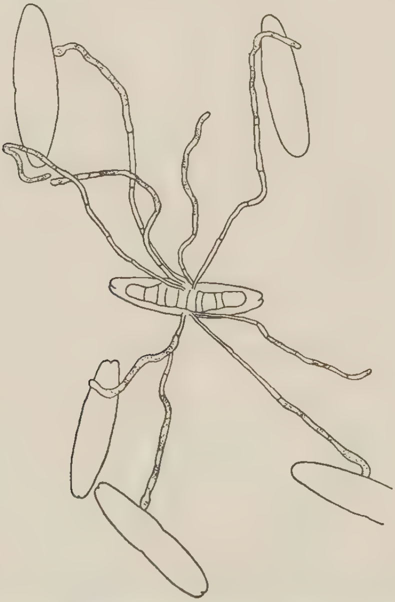

Fig. 5. *Ancylistes* sp. in *Tetmemorus granulatus*. Infection of new host cells by external hyphae. × 150.

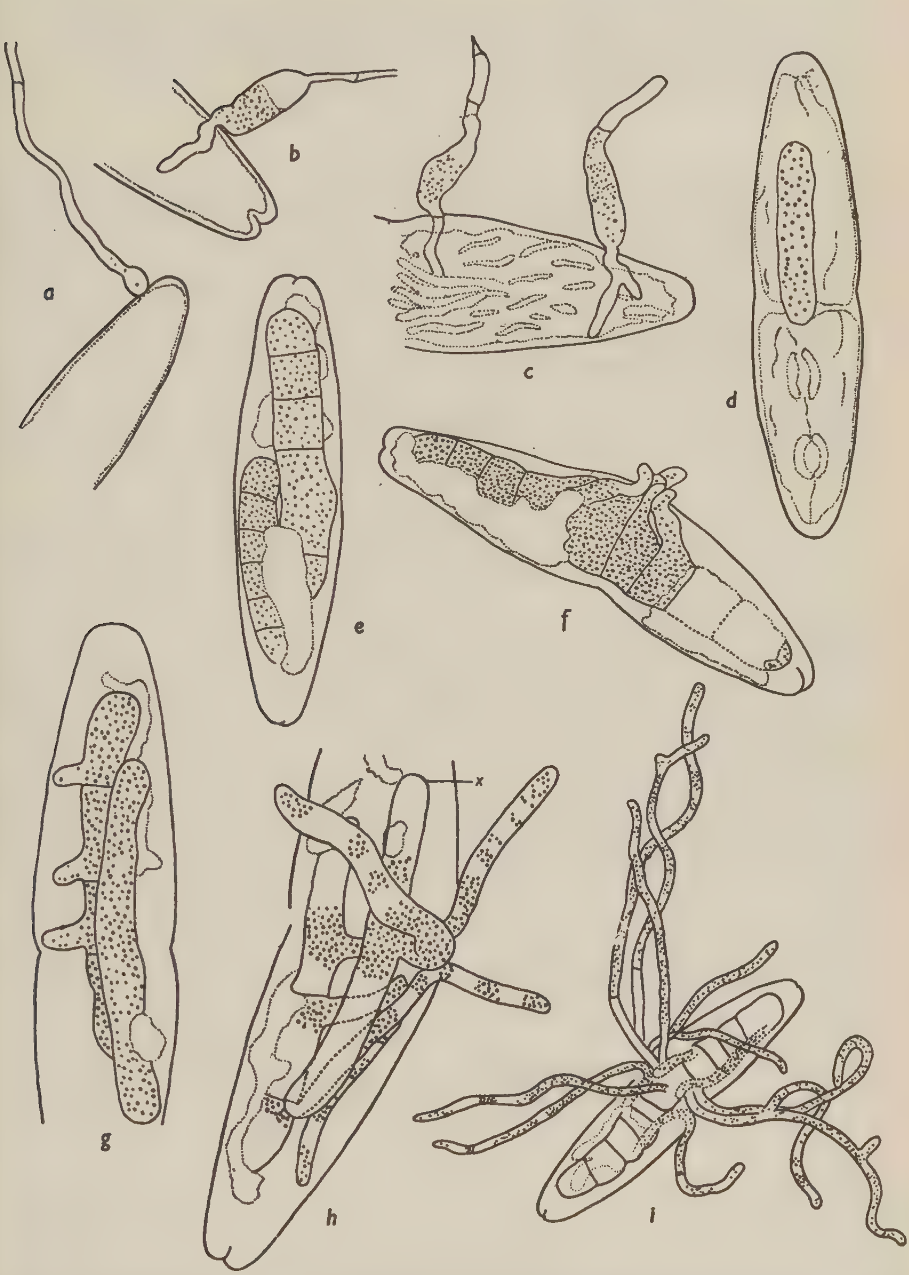

Fig. 6. *Ancylistes* sp. *a–c*, early stages in infection by external hyphae. *d*, young mycelium. *e*, two mycelia which are divided into segments. *f*, *g*, mature mycelia producing external hyphae. *h*, non-septate thallus at *x* producing a single broad external hypha. *i*, empty mycelium with growing external hyphae. *a–h*, × 500; *i*, × 300.

ovoid ball-like structure formed by the rapid entrance of the protoplasm of the swollen external infection cell into the desmid was not observed. It is only when further stages in the life history of this fungus are known that its affinities can be discussed.

Summary

Aphanomycopsis bacillariacearum has been found in *Pinnularia* sp. from Blelham Bog, near Wray Castle, and a new species *Aphanomycopsis desmidiella* is described parasitizing the desmid *Netrium digitus*. *Ancylistes* is recorded for the first time from Great Britain and the presence of a conidial stage is confirmed. *Tetmemorus granulatus* Bréb. is a new algal host for this parasite and the emergence of external hyphae at the junction of the two semi-cells of the desmid has not formerly been described.

My grateful thanks are due to Miss E. M. Wakefield of Kew, for the Latin diagnosis, and to Prof. C. T. Ingold for reading the manuscript.

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NOTES ON TWO STRAINS OF *FUSARIUM OXYSPORUM* FROM THE OIL PALM IN THE BELGIAN CONGO

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(With Plate XVII and 6 Text-figures)

INTRODUCTION

The two strains of *Fusarium oxysporum* considered here were isolated from the Oil Palm (*Elaeis guineensis*) by Prof. C. W. Wardlaw and identified by Dr W. L. Gordon of the Dominion Laboratory of Plant Pathology, Winnipeg (Wardlaw, 1946*a*, *b*). One of these strains was associated with a wilt disease characterized by necrotic discoloration of the vascular strands of the trunk and roots in palms of four to twenty years of age. The other strain was isolated from a characteristic leaf disease known as 'patch yellow', certain genetical types of palm being apparently highly susceptible. As noted above, both strains have been identified as forms of *Fusarium oxysporum*.

Four isolations from necrotic vascular strands and three from diseased leaves were available for study. Preliminary investigations indicated that the several isolations of each group were identical, but the two groups described here as *Fusarium* W (from wilt) and *Fusarium* P (from 'patch yellow' leaf disease), though closely comparable in some respects, were different in others. On some media, for example, the two strains look very much alike. Among the writer's aims, therefore, was to find a synthetic medium (or media) which (i) would serve to distinguish the two strains, and (ii) would be suitable for the manifestation of the principal morphological characters of the strains.

In this paper an account is given of preliminary mycological investigations of the two strains. The investigation of diseases of the oil palm, an important source of vegetable fats and oils, is still at an elementary stage. Although the pathogenicity of the two strains to be considered here has yet to be established, it is a matter of much interest that *F. oxysporum*, the causal organism of many important tropical diseases, has been observed associated with two very different and apparently unrelated pathological conditions in the oil palm.

METHODS

The experiments were based on single conidial cultures. No sectoring was observed in either of the strains.

As one of the main objects was to find a medium or media which would serve to distinguish the two strains, a large number of different media and variants thereof were used, including malt agar, Czapek-Dox agar, modifications of Brown's synthetic agar, Sabouraud's agar, and potato agar.

Conidial suspensions in sterile physiological saline solution were used for the inoculation of Petri dish cultures. The densities of the conidia in the suspensions were adjusted after haemocytometer counts had been made; the plates were inoculated with a platinum loop.

Observations were made on the appearance of cultures, the development of colour in the medium, rate of linear growth, size and shape of conidia, occurrence of chlamydospores, etc. In general, the experiments were carried out in triplicate at 24° C.

Observations

General. On the several different media used the rate of linear growth was practically identical in the two strains. On most of the media the two strains were not identical in appearance, but the differences between them were often slight. Some media, however, brought out quite definite differences. Characters in which marked contrasts were observed in different media included (*a*) pigmentation, and (*b*) amount of aerial mycelium. These were recorded in cultures two weeks old and again after four weeks, the two sets of observations being closely comparable. The colours (Ridgway, 1912) shown by the strains on different media are set out in Table 1.

Characterization of strains on a standard medium. In the light of much preliminary experimental work I eventually selected the following modified recipe of Brown's synthetic medium for this purpose: glucose, 2 g.; potato starch, 25 g.; sodium nitrate, 2 g. (asparagin was not available); tri-potassium phosphate, 1·25 g.; magnesium sulphate, 0·75 g.; agar, 15 g.; water, 1000 ml. Media with 5, 7·5 and 10 % potato starch were also found to be satisfactory for normal growth. The data obtained with the selected standard medium after two weeks are set out in Table 2.

The mycelial growth of strain W was thinner in modified Brown's synthetic medium with lower concentrations of potato starch, although it was more dense than that of strain P. The pigmentation produced by the strains when different concentrations of potato starch were used varied: it became deeper as the concentration of starch was increased (Pl. XVIII, figs. 1–4). In comparable cultures of the two strains the pigmentation developed earlier in strain P than in strain W, but later the pigmentation in strain W was deeper than in strain P.

In strain P both the total sporulation and the proportion of microconidia to macroconidia were increased as the concentration of potato starch in the medium was decreased, i.e. septation increased with increase in starch concentration. Microconidia were predominant in strain W over the whole range of starch concentrations tested. Chlamydospores were abundant in strain W in the lower concentrations of potato starch, and also appeared in higher concentrations as the cultures became older. Chlamydospores were not observed in strain P in the higher starch concentrations. In general, the conidia of strain P show much more curvature than those of strain W (Text-figs. 1 and 2). This is at once apparent when the two strains are cultured on potato blocks.

From these observations it appears that strain P responds more readily to changes in the medium than does strain W.

Growth on modified Brown's synthetic medium with different concentrations of glucose. When glucose was used in place of potato starch a very light pink colour was produced by both strains at the several concentrations after two weeks. At higher concentrations of glucose the colour gradually changed to reddish purple and finally 'claret red'. The 'indigo blue' colour observed in media containing potato starch was not observed. It can therefore be concluded that the 'indigo blue' colour in the medium is associated with the breakdown of the starch into sugar.

Table 1. *Colours (Ridgway) produced on different media*

Medium	Strain W	Strain P
Malt agar	Pale 'pinkish buff'	'Deep hellebore red'
Sabouraud's agar	No characteristic coloration	'Rocellin purple'
Potato agar	Pale 'drab grey'	'Smoke grey'
Rice (whole)	Light pink becoming 'shell pink'	'Claret red' becoming 'Pompeian red'
Brown's synthetic medium (modified with rice starch)	Light pink; later becoming 'indigo blue' at higher rice concentrations	Light pink; later becoming 'indigo blue' at higher rice concentrations
Czapek-Dox agar	No characteristic coloration	No characteristic coloration
Brown's synthetic medium (modified: no potato starch)	No characteristic coloration	No characteristic coloration

Mycelial growth in strain W was stronger than that in strain P in all the media used except the synthetic media which gave very thin growth.

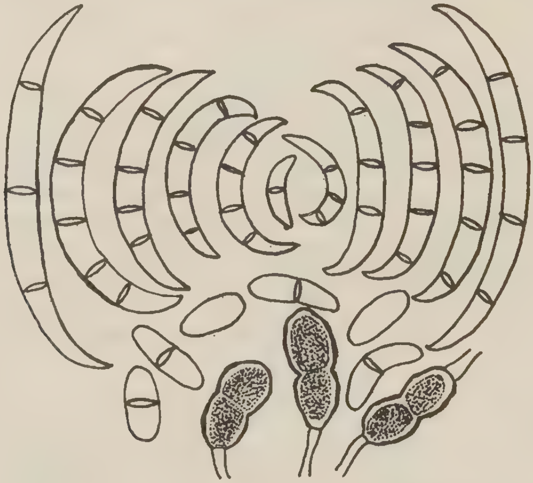
Table 2. *Characters of strains on the standard medium*

	Strain W	Strain P
Mycelium	Luxuriant aerial mycelium	No aerial mycelium: pionnotal development
Pigmentation of medium (after two weeks)	'Indigo blue' colour in patches near centre extending towards periphery*	Ring of 'indigo blue' colour extending from about 2 cm. from centre towards periphery (see Pl. XVIII)
Pigmentation of medium	Entire culture 'dull violet black' (after four weeks)	'Flesh pink' colour associated with whole culture; 'indigo blue' colour more marked
Sporulation (based on 500 conidia)†	Microconidia approx. 60 %	Microconidia approx. 30 %
Septation of macroconidia	Macroconidia from 1- to 7-septate; 1- to 5-septate conidia predominant; range of length of 3- to 7-septate conidia, 15 to 54 μ	Macroconidia from 1- to 9-septate; 1- to 5-septate conidia predominant; range of length of 3- to 9-septate conidia, 15 to 39 μ
Curvature of macroconidia	Less curved	Much curved, becoming sickle-shaped
Attachment of conidia	Microconidia and macroconidia directly abstricted; no secondary branches (Text-fig. 5)	Microconidia abstricted from tips of short secondary branches in rapid succession, accumulated and formed into spherical heads; macroconidia not in heads (Text-fig. 6)
Chlamydospores	Abundant	Very few

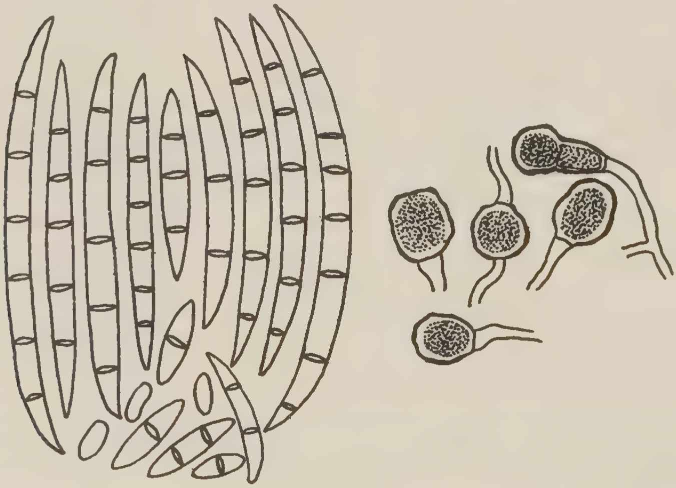
* N.B. When strain W was cultured on this standard medium several months later, the blue colour did not develop in the standard medium containing 2·5 % starch, but developed slowly in the higher starch concentrations. This is now known to be due to the replacement of the wild type by mutant forms.

† Conidial samples were taken 1 cm. from the centre of cultures two weeks old.

Growth on modified Brown's synthetic medium with different concentrations of rice starch. Similar results were obtained when the two strains were grown on modified Brown's synthetic medium with different concentrations of rice starch. A light pink colour was produced by both of the strains over the whole range of concentrations. 'Indigo blue' colour was observed in the media with higher starch concentrations after two weeks. The amount of sporulation and the septation of conidia in the two strains on these rice media were closely comparable with those observed in media containing potato starch. But in the matter of curvature, whereas at low concentrations of rice starch (0·05 %) strain P showed greater curvature than strain W, this was reversed at high concentrations (15 %).



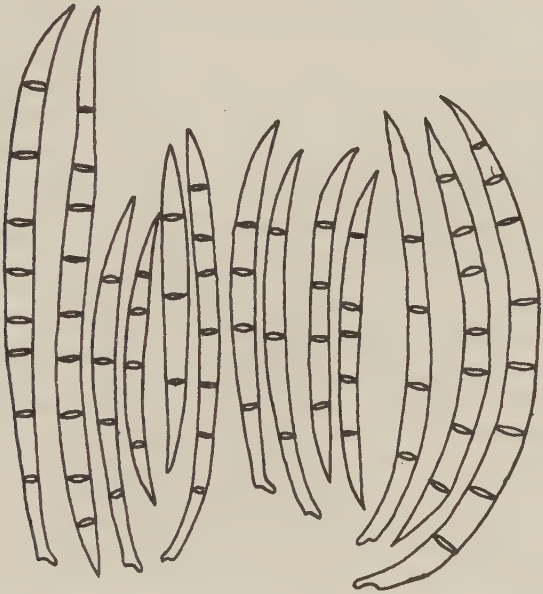
Text-fig. 1. A group of conidia and chlamydospores of strain P. (× 1000.)



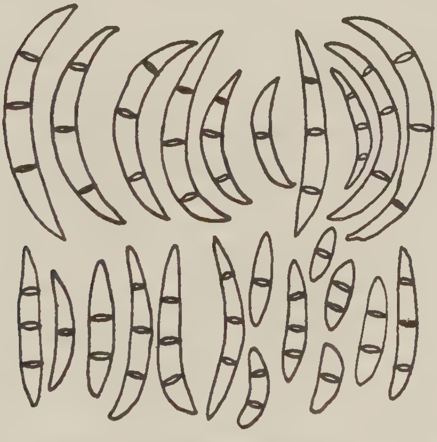
Text-fig. 2. A group of conidia and chlamydospores of strain W. (× 1000.)

Growth on soil. As strain W was associated with a vascular wilt disease, and therefore presumably soil-borne, the investigation was extended by growing both strains on a sterilized loam soil.

In each instance the fungus spread slowly over the surface of the soil and down into it. Microscopic preparations of the aerial mycelium after two weeks showed that macroconidia were predominant in strain W. They were very long and from 16·9 × 2·8 to 70·3 × 3·4 μ; 4- to 8-septate conidia were common (Text-fig. 3). Macroconidia and microconidia were present in equal proportions in strain P (Text-fig. 4). The macroconidia of strain P measured from 8·4 × 2·8 to 33·7 × 3·4 μ. They were of different sizes and shapes. The macroconidia were from 1- to 6-septate, 1- to 3-septate conidia



Text-fig. 3. A group of macroconidia of strain W showing high septation. (× 1000.)

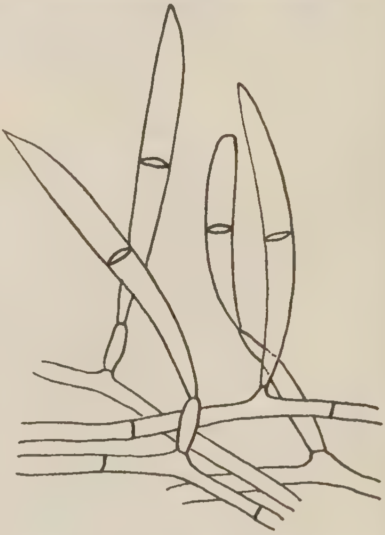


Text-fig. 4. A group of macroconidia of strain P. (× 1000.)

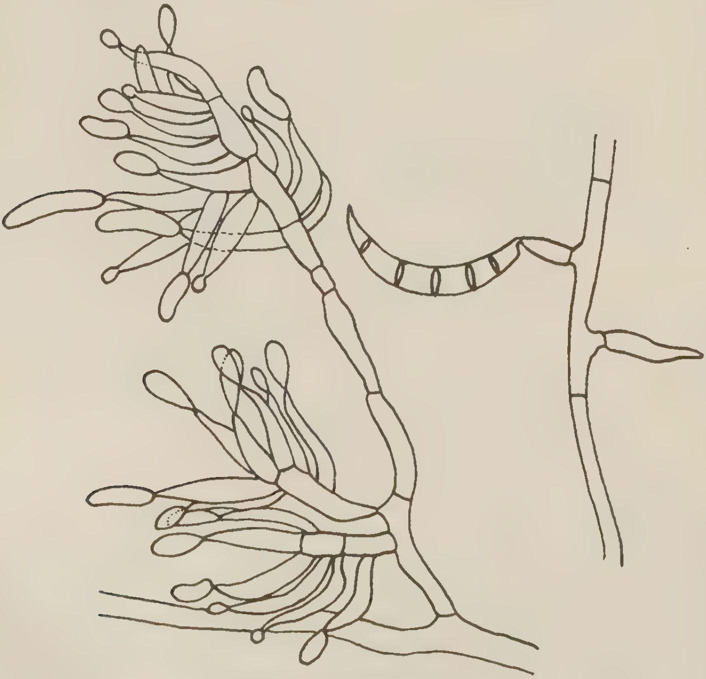
being common. Some of the highly septate conidia were typically sickle-shaped and with well-defined apices and pedicels. On starch-containing media, as described in the previous section, the conidia of strain P were larger than those of strain W. On this soil medium the reverse was found. In both strains only surface mycelium yielded conidia.

The strains were grown on an aqueous extract of the loam stiffened with agar, the pH being adjusted to 5·6. Probably this medium was too dilute, for only a very thin non-sporulating mycelium was obtained.

Effect of light and darkness on the growth of the strains. A striking difference was noted when the two strains growing under comparable conditions were exposed to light and darkness on a synthetic medium containing glucose, potato starch and the usual mineral ingredients. Strain P consistently showed zonations in those cultures which had been exposed to the light and an absence of zonation in those in the dark; but strain W showed no zonation under either condition.



Text-fig. 5. Conidia and conidiophore of strain W. (× 1000.)



Text-fig. 6. Conidia and conidiophorein of stra P. (× 1000.)

DISCUSSION

The observations show that the two fungi, which have been identified as strains of *Fusarium oxysporum*, though closely comparable in some respects, are not identical. Thus, on the same media, differences were observed in the pigmentation produced in the medium, in zonation when exposed to light, in the amount and appearance of the mycelium, and in sporulation. Furthermore, under certain cultural conditions, the two strains showed appreciable differences in the taxonomically more important characters of size, shape, septation and manner of abstriction of their conidia. These characters carry much weight, especially in the system of Wollenweber and Reinking (1935). On some media, however, the two strains were very much alike, for example, on modified Brown's synthetic medium and on Czapek-Dox's medium. It has been found convenient to select a particular medium as a standard medium: this serves to show the contrasted characters of the two strains. Actually, by suitably modifying media, the two strains can be made to approximate very closely in their morphological and physiological characters.

A difference of marked interest was noted when the two strains, grown under comparable conditions, were exposed to light and darkness. Strain P showed zonation in those cultures which had been exposed to light. This observation confirms those of Snyder and Hansen (1941 *b*), who recognized the fact that light is the only one factor in the environment which may cause zonation. But strain W showed no zonation whatsoever.

The results of this investigation are of interest in relation to previous work on the classification of the genus *Fusarium*. The two strains can be separated by several morphological characters of the kind used and considered valid by Wollenweber and Reinking (1935). But, according to Snyder and Hansen (1940, 1941 *a*, 1945), these morphological characters are unstable and unreliable as taxonomic criteria. These authors also consider that the species of *Fusarium* which cause wilt diseases are merely biologic forms of one and the same species, i.e. *F. oxysporum*. In short, the several forms or strains are to be recognized by their specific pathogenicities. As a practical issue, it should, however, be noted that much time will probably elapse before the relevant experiments on pathogenicity can be carried out with the oil palm. In the meantime it may be anticipated that, as this disease becomes more fully investigated in different regions, further isolations of *Fusarium* will be made. If so, it will clearly be desirable to have some standard with which these new isolations can be compared. Hence, the importance of a knowledge of the morphological and physiological characters of the strains so far obtained.

Studies of the pathogenicity of the two strains, strain P on leaves, and strain W as affecting the vascular system, will be awaited with interest. Reciprocal inoculations will clearly be both of academic interest and practical importance. Indeed, it is probably rather unusual to find two strains of an organism like *F. oxysporum* associated with such very different manifestations of disease as in the present instance.

SUMMARY

1. An account is given of two strains of *Fusarium oxysporum* which were isolated from leaves and from diseased vascular strands of the oil palm (*Elaeis guineensis*) in the Belgian Congo.

2. An account is given of the growth, colour reactions and sporulation of the two strains on a selected synthetic culture medium. Other cultural tests by which the strains may be recognized and compared are also indicated.

3. When the two strains were grown under comparable conditions differences were observed in pigmentation, in zonation when exposed to light, in the amount and appearance of mycelium, and in sporulation; but media could be selected in which the two strains appeared identical.

4. The relation of these results to taxonomic aspects and future work on such *Fusaria* as may be found associated with these diseases of the oil palm is discussed.

The writer wishes to express her thanks to Prof. C. W. Wardlaw for suggesting this work, and for his helpful criticism during its progress, and to Mr E. Ashby for the preparation of photographs.

Postcript. After this manuscript was sent to press, it was found that the wild type of strain W is subject to mutation, though not of the sectorial type. It is now known that the large macroconidia shown in text-figure 3 are of mutant type. These matters will be described in a later paper.

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EXPLANATION OF PLATE XVII

Figs. 1, 2. Cultures of strain W in modified Brown's synthetic medium with 2·5 and 10 % potato starch respectively, showing luxuriant mycelial growth and development of 'indigo blue' colour.

Figs. 3, 4. Cultures of strain P on same media showing scanty mycelial growth and development of 'indigo blue' colour.

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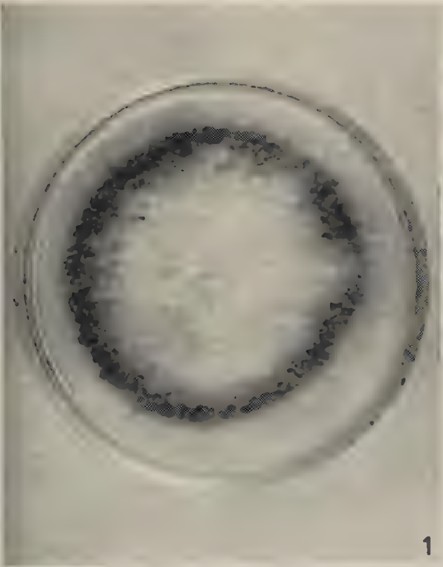
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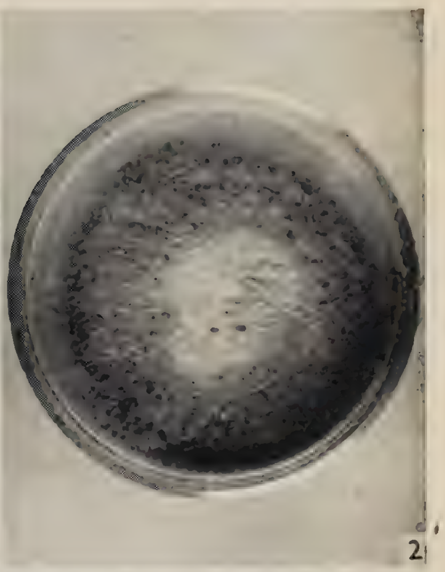
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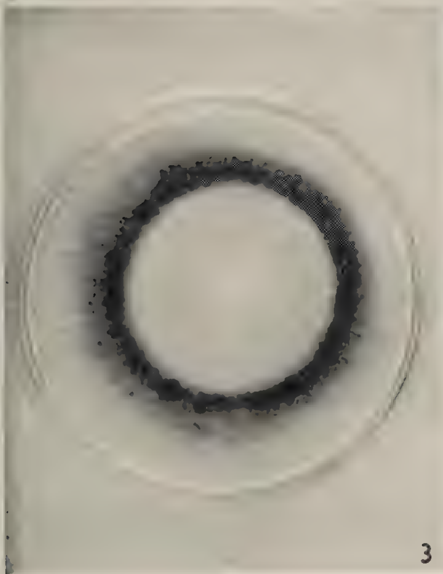
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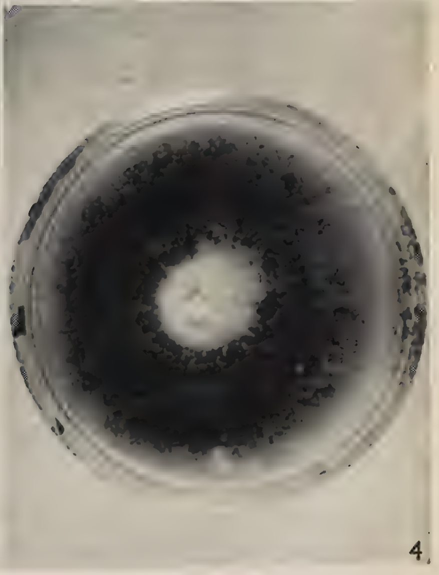
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THE ECOLOGY OF FUNGI WHICH CAUSE ECONOMIC PLANT DISEASES*

By A. S. BOUGHEY, *University College, Exeter*

(With 2 Text-figures)

Ecological investigations on fungi have not developed on anything approaching the scale of those on higher plants. They have been confined chiefly to studies on terricolous Agarics, where it has been shown (Wilkins, Ellis & Harley, 1937; Grainger, 1945) that distribution is determined by the nature of the substrate, this depending on the character of the subsoil, the type of higher plant dominants and ground cover, and climatic factors such as temperature and moisture.

At the same time there has been a vast increase in the number of plant pathological papers. It has been estimated (Foister, 1946) that some 10 % of this literature is concerned with ecological investigations on particular organisms. The plant pathologist is now in fact compelled to study not only the etiology of a disease but also the epidemiology of its causal organism. The data on the autecology of individual fungi thus accumulating will ultimately prove invaluable when a comprehensive ecological review of the whole group is attempted. In the meantime it is interesting to examine whether synecological data may be extracted from such work on the regional distribution of fungi as has already appeared.

DIFFICULTIES OF SYNECOLOGICAL FUNGAL STUDIES

There are several immediate difficulties inherent in any synecological study of fungi. First, as fungi are not free-living organisms, their distribution is restricted to the geographical range of their particular host or substratum. As a result of this dependence on living or dead organic matter, the relationship between individuals and between species is less direct than that found in a higher plant community. In a closed community of higher plants both individuals and species compete, primarily for light, water, and nutrient salts. Such a community of directly competing individuals and species is less common among fungi, occurring chiefly in the soil, where the phenomenon of competition is called antagonism, and among wood-invading and aquatic fungi. A third difficulty arises because a considerable part of the life history of a fungus may be spent within another living or dead organism, where the physical and chemical features of the environment are not easily measured.

SYNECOLOGICAL STUDIES ON FUNGAL PATHOGENS

These difficulties may to some extent be avoided, as was first pointed out by Butler (1925), by considering only those fungi which cause diseases of *cultivated* plants. Wild species of higher plants are usually restricted to a narrow range of habitats, those in which they can maintain themselves against competition from other wild plants. Their fungal parasites are therefore of necessity restricted to the same narrow range. Parasites of cultivated plants, on the other hand, suffer no such narrow limitation, for cultivated plants are often grown under a very wide range of climates and soils. It is therefore possible to examine the behaviour of these fungi under widely contrasted environments.

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* Paper presented to Section K, Brit. Ass. for Adv. Sci. 1947.

Environment will react strongly on a parasitic fungus only at certain phases of its existence. For the most part the fungus will be growing within its host, in a constant environment modified only by relatively small changes resulting from interaction between the host and its habitat. To be a successful parasite of a cultivated plant, a fungus must not only be able to establish itself and live within this constant host environment, but must survive the periods when it is exposed outside its host. There are usually three such periods. The first occurs during long-distance dispersal, for to be a successful parasite the fungus must have some effective method of spreading itself widely, to become ubiquitous. The second is during out-of-season survival, for there are few agricultural areas of the world not subjected to periods of excessive heat or cold, or severe drought or flood. The last and most important period of all will be during short-distance dissemination, when the fungus is spreading rapidly and locally, enabling the disease to reach epiphytotic proportions. This dissemination stage is as vital in the development of a fungal pathogen as the seedling stage is in the higher plant, and it is therefore on this stage that environment will have its greatest effect.

Short-distance dissemination types

The fungi which cause diseases of cultivated plants can be conveniently classified into seven major groups according to their method of short-distance dissemination: Powdery Mildews, Downy Mildews, Rusts, Smuts, Fungi Imperfecti, Pyrenomycetes, and Hymenomycetes. These groups are taxonomic units, not all of the same status, which have been defined in the first place on the characters of their sexual spore stages. Nevertheless, each group shows a remarkable uniformity in its method of short-distance dissemination.

Powdery mildews are disseminated by means of air-borne conidia. These are short-lived, rarely surviving more than forty-eight hours, and in most instances so far investigated appear to be capable of germinating in air of any atmospheric humidity, but only with difficulty in water (Berwith, 1936; Yarwood, 1936; Hashioka, 1937; Longree, 1939; Stoll, 1941; Brodie & Neufeld, 1942; Cherewick, 1944).

Downy mildews are disseminated by means of sporangia, in a few instances reduced to conidia. The sporangia appear to require the presence of free water for some three to five hours before they can germinate, liberate their zoospores, and establish a fresh infection (Müller, 1930; Richards, 1939; Magie, 1942; Yarwood, 1943).

Rusts are disseminated, as far as those causing diseases of cultivated plants are concerned, principally by means of uredospores. These are relatively long-lived spores, remaining viable usually for some two or three months, and requiring, in such as have been examined, the presence of free water or a saturated atmosphere before they will germinate (Beauverie, 1924; Goldsworthy & Smith, 1931; Hemmi & Abe, 1933; Yarwood, 1939; Straib, 1940).

Smuts are usually disseminated by means of chlamydospores, which are long-lived, but their mode of infection is not so uniform as in the other six groups. It is apparently assumed that these chlamydospores require free water to bring about germination, for there seems to be no record of investigations on their germination in the dry state.

Fungi Imperfecti have been shown to fall into two groups, according to whether their conidia can be scattered dry by air currents or must first be wetted (Mason, 1937), but conidia of both types apparently require free water, or a relative humidity of over 90 %, before they will germinate (Barrus, 1921; Stevens, 1932; Dennis & O'Brien, 1933; Vestal, 1933; Wellman, 1934; Inoue, 1937; Katsura, 1937; Burke, 1938; Guba, 1938). It is possible that wetting with water is always an essential preliminary to germination, as was shown for *Diplocarpon rosae* by Lyle (1938).

From the plant pathological point of view Pyrenomycetes form an unsatisfactory group, for their perfect stages are often saprophytic or rare, and can play no major part in the short-distance dissemination of the parasite. For ecological studies it is possibly better to include the Pyrenomycetes under Fungi Imperfecti, but for present purposes they have been treated as a separate group.

Hymenomycetes are disseminated by air-borne basidiospores. Like the chlamydospores of smuts these basidiospores are apparently always assumed to require the presence of free water or a nutrient solution before they will germinate.

Short-distance dissemination will, however, involve not only the question of spore germination but also that of spore production. This appears to be influenced by water and air humidity in much the same way as spore germination. Thus, production of conidia by powdery mildews is depressed in the presence of free water or very high atmospheric humidities (Hashioka, 1937), while that of sporangia in downy mildews (Weston, 1923; Yarwood, 1937, 1943; Ogilvie, 1944; Beaumont, 1947) and conidia in Fungi Imperfecti (Petri, 1931) is favoured.

It appears also that successful infection is dependent on much the same moisture conditions as lead to optimum spore germination, for it takes place most readily under dry conditions with powdery mildews (Yarwood, 1936; Hashioka, 1937), under wet conditions or in high atmospheric humidities with downy mildews (Schultz, 1937; Ogilvie, 1944), rusts (Fromme, 1913; Lauritzin, 1919; Goldsworthy & Smith, 1931; Asuyama, 1939), and Fungi Imperfecti (Muncie, 1917; Lauritzin, 1919; Barrus, 1921; Dastur, 1921; Van der Muyzenberg, 1932; Stahel, 1937; Burke, 1938).

It is therefore not surprising to find that outbreaks of disease epidemics have been correlated with the occurrence of rainfall or dews in downy mildews (Salmon & Ware, 1931; Crosier & Reddick, 1935; Moore, 1937; Schaal & Edmundson, 1943), rusts (Johnston, Melchers & Miller, 1938; Belin, 1938; Matheny, 1939; Melchers & Johnston, 1939; Craigie, 1945) and Fungi Imperfecti (Barrus, 1921; Dastur, 1921; Elliott, 1922; Stevens, 1932; Wellman, 1934; Guba, 1939; Hendrickx, 1942). Moreover, as the presence or absence of free water or high humidities has this controlling influence over the short-distance dissemination phase of fungal pathogens of cultivated plants, some degree of correlation may be expected between rainfall and the geographical distribution of economic plant diseases.

Distribution of Sudan plant diseases

The diseases of cultivated plants in the Anglo-Egyptian Sudan have been recorded on a regional basis (Boughey, 1946) to facilitate subsequent ecological analysis. It has been shown (Boughey, 1947) that the distribution of the pathogens (fungi, bacteria, and viruses) which cause these diseases is positively and significantly correlated with the amount of annual rainfall. The number of diseases in the wettest areas is approximately three times that found in the arid ones.

It is interesting to examine the distribution of the Sudan fungal pathogens in each of the seven groups previously described (Fig. 1*a*). Powdery mildews predominate in the arid areas, gradually decreasing with increasing rainfall to reach a very low percentage representation in the wettest areas. With Fungi Imperfecti the exact opposite is found, diseases caused by this group predominating in the wettest areas, then decreasing, until they are almost completely absent from the driest area. Downy mildews tend to increase with increasing rainfall, while rusts first decrease then increase. Other groups, including the miscellaneous fungi belonging to unclassified groups, show no consistent variation.

The system of disease recording just described has not been used in any other country. It is not therefore possible to compare the Sudan results, as regards fluctuations in the total number of pathogens, with those from other sources. The method of analysing the composition of the plant-disease flora, on the other hand, can be applied to any comprehensive plant-disease list, and direct comparison made.

Distribution of Scottish plant diseases

A list of Scottish plant-disease records on a regional basis has been produced by Dennis and Foister (1942). As the regional basis for their list they used a previous subdivision of Scotland into twelve main drainage areas. Five of these areas, Orkney, Sutherland, Ross, Hebrides, and Argyll, have too few records for them to be included in any analysis. The other seven can be divided into three groups, a low rainfall east-coast group (Moray, Dee, Forth, and Tweed), a high rainfall west-coast group (Clyde and Solway), with Tay having an intermediate rainfall.

The percentage representation of each of the seven classes of fungal pathogens in each of these three rainfall groups is shown in Fig. 1*b*. Much the same trends can be observed as with the Sudan data in Fig. 1*a* with the addition that smuts and Pyrenomycetes also appear to decrease with increasing rainfall. The trends are not nearly so marked as with the Sudan data, but the rainfall differences are very much smaller.

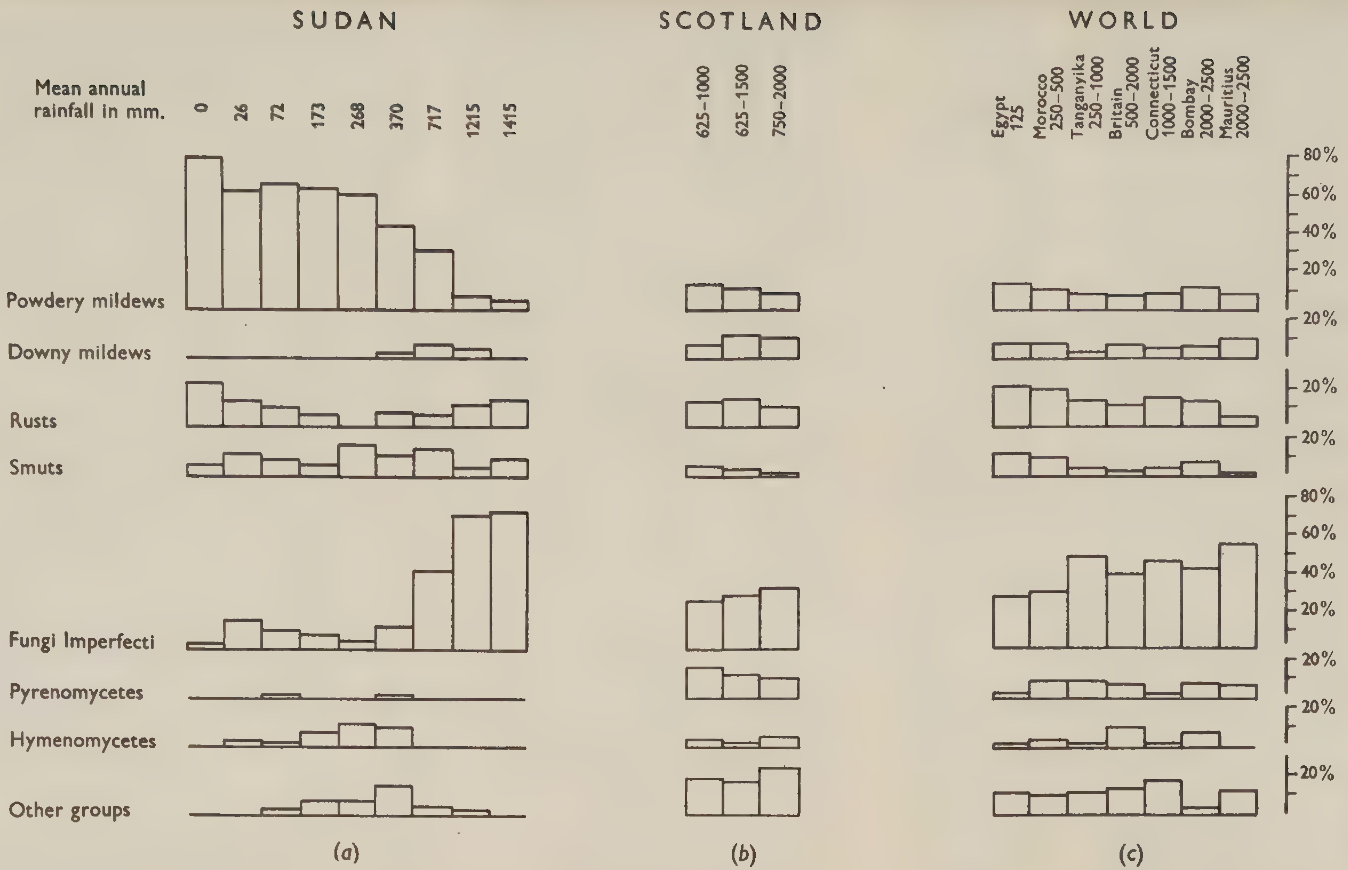

Fig. 1. Proportional representation of fungal groups in (*a*) nine districts of the Sudan, (*b*) three areas of Scotland, (*c*) seven scattered countries

DISTRIBUTION DATA FROM A REPRESENTATIVE SERIES OF PLANT-DISEASE LISTS

Instead of considering data from individual countries such as the Sudan or Scotland, the plant-disease lists of scattered countries may be used, provided that these countries from fairly uniform areas and that their lists are reasonably complete. Lists from seven areas were selected as meeting with these requirements, and providing a sufficient contrast in the amount of annual rainfall. These lists were for Egypt (Reichert, 1921), Morocco (Maire & Werner, 1937), Mauritius (Shepherd, 1937), Tanganyika (Wallace, 1937), Britain (British Mycological Society, 1944), the Bombay province of India (Uppal, Patel & Kamat, 1934), and the Connecticut State of the U.S.A. (Clinton, 1934).

The relationship between the mean annual rainfall and the composition of the plant-disease flora of these areas is shown in Fig. 1*c*. Comparing it with the Scottish list illustrated in Fig. 1*b*, the same general trends can be discerned although, as must be expected with lists from such varied sources, there are a number of anomalous points.

DISCUSSION

It has not been found possible to provide this present examination of plant-disease floras with a statistical basis. Nevertheless, the existence of similar trends, in data from three quite different sources, affords some considerable support for the existence of a real relationship between the annual rainfall of a given area and the proportion of fungal pathogens in each of the seven major groups which have been described. It appears that this proportion can be calculated from the annual rainfall alone, without reference to any other factor such as air temperature. Thus a temperate area, such as the Solway-Clyde area of Scotland, may have much the same group representation as a tropical island like Mauritius, which has something approaching the same mean annual rainfall in its agricultural areas. This similarity is illustrated in Fig. 2*a*. That is not to say, of course, that *individual* fungal species will be unaffected by temperature, for temperature is often quite clearly the main factor determining *specific* distribution.

The simplest explanation for this relationship between group distribution and annual rainfall may perhaps in some instances be the correct one. If powdery mildew conidia are not produced and do not germinate abundantly in the presence of free water, powdery mildews would be less numerous in countries with a high rainfall. Similarly, if water is necessary for the production and germination of the sporangia of downy mildews and the conidia of Fungi Imperfecti, it is not surprising to find these two groups better represented in the higher rainfall areas. With this simple explanation, however, rusts and smuts should increase with rainfall too, while actually they decrease. With rusts, perhaps some host factor comes into operation, or possibly the heavy dews which are so characteristic a feature of arid agricultural areas may replace rainfall. Such an explanation would satisfactorily account for the distribution of rusts in the Sudan, where rusts are best represented in both the wettest and driest districts. The relationship between smuts and their environment is bound to be a more complex one because of their varied methods of establishing infection. Whatever the true explanation for the relationship, it lies beyond the scope of the present paper, which is concerned merely with the demonstration of the relationship.

Given this rainfall-distribution relation, it is possible to construct a typical profile for an arid area, and for a very wet area, areas of intermediate rainfall having correspondingly intermediate profiles (Fig. 2*b*). The arid profile, obtained by combining the disease lists from the arid provinces of the Sudan and Egypt, is characterized by a great predominance of powdery mildews, a fair representation of rusts, smuts, and Fungi Imperfecti, with low numbers of downy mildews, Pyrenomycetes, Hymenomycetes, and miscellaneous fungi. The wet profile, obtained by uniting Mauritius and the Solway-Clyde area, has a very high percentage of Fungi Imperfecti, increased representation of downy mildews, Pyrenomycetes, and miscellaneous fungi, with lower numbers of powdery mildews, rusts, and smuts, and still low figures for Hymenomycetes.

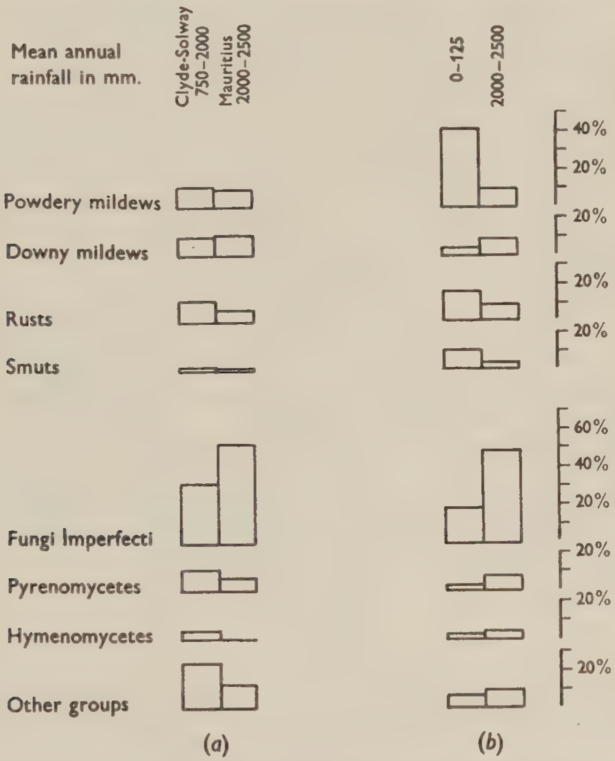

Fig. 2. Proportional representation of fungal groups in (*a*) two areas of approx. similar rainfall, (*b*) hypothetical very dry and very wet areas.

It should be possible to find some better measure of the amount and duration of the wetting with free water to which crops will be subjected than annual rainfall. The total number of days in the year when rain falls or dew is deposited, for instance, might provide a better assessment of this wetting. Also, in this paper no attention has been paid either to annual variations in the distribution of the fungi which cause diseases of cultivated plants, or to fluctuations in the *incidence* of such parasites. While both are directly related to, and will have an important effect on, the regional occurrence of plant diseases, it is believed that their proper consideration will not radically change the general conclusions as to fungal ecology which have been presented.

There are several implications arising from this ecological study in regard to agricultural and horticultural practices. First, the greatest proportion of seed-borne diseases of economic plants belong to the Fungi Imperfecti. As this group is most strongly represented in wet areas, which appear to have in any event larger numbers of diseases than dry ones, seed production, from a plant pathological point of view, should be concentrated in the driest parts of a country. The United States administration has already made use of this suggestion, but other countries appear slow to follow suit. It is true that there is a concentration of seed production in the eastern counties of England, but this appears to have arisen for other reasons. Secondly, when rainfall is supplemented by irrigation, the method of applying the water is of some plant pathological importance. Where overhead irrigation, instead of flooding or subsoil irrigation, is used, a wet type of climate will be simulated in an otherwise dry area, and troublesome diseases not normally present in the area may occur. Where overhead irrigation must be used, a fungicide could perhaps be added to the water. The third point is rather similar. Plant-house watering should be arranged as far as possible to avoid wetting foliage.

In conclusion, while it seems that lists of economic plant diseases can be analysed to provide data on the synecology of fungi, their value in this respect would be greatly increased if variable areas could be subdivided into districts showing some uniformity, and if diseases which are absent, as well as those which are present, could be recorded.

Summary

There have been comparatively few synecological studies on fungi, possibly because difficulties arise from the dependence of fungi on living or dead organic matter, the frequent absence of competition, and the problem of assessing habitat factors. By considering only those fungi parasitic on cultivated plants, these difficulties can be partially avoided.

An analysis of Sudan plant disease records shows that the number of pathogens increases with increasing rainfall, powdery mildews predominating in arid, Fungi Imperfecti in wet areas. Similar trends can be detected in Scottish records, and in a set of disease lists from seven representative countries.

The *proportion* of powdery mildews, downy mildews, rusts, smuts, Fungi Imperfecti, Pyrenomycetes, Hymenomycetes, and the remaining fungi in an economic plant disease flora can apparently be estimated from annual rainfall alone.

Implications regarding agricultural and horticultural practices are suggested.

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PROCEEDINGS

MYCOLOGICAL EDUCATION

Meeting held in the Department of Biology, Chelsea Polytechnic, London, at 11.0 a.m., 31 January 1948. The President, Prof. A. E. Muskett, in the Chair.

G. C. Ainsworth. The Society and mycological education.

Although the British Mycological Society does not profess to be an educational organization, education is implicit in many of its activities. During the past fifty years the Annual Autumn Forays have played a major role in training successive generations for the study of our larger fungi and in maintaining the so-called 'tradition of species'. Since 1918, the Plant Pathology Committee has done much towards educating plant pathologists to treat the names of plant pathogenic fungi with greater respect and understanding. But every member, whether professional or amateur, who has made any progress with a study of mycology would probably have to admit a greater or less debt to the Society—and there are very few British mycologists whose names have not appeared in the Society's membership lists.

About five years ago the Society felt concern at the state of systematic mycology in this country and the Council appointed a Committee to investigate the position and make recommendations. In June 1944 the committee's report was printed (but not published), and in the following month copies were distributed to government departments, universities, research stations, and other bodies interested in mycology.

The work of preparing that report raised the question of the training of much needed systematists. At the same time the war had emphasized the importance of mycology in diverse fields and focused attention on the absence of educational facilities for those wishing to take up such mycological work. The Council therefore set up a second committee to make recommendations regarding the teaching of mycology. Much valuable information was derived from a questionnaire sent out to all university departments, agricultural colleges, and other teaching institutions known to be interested or potentially interested in some aspect of mycological teaching and the committee's report, like the earlier report, was printed but not published and widely circulated in June 1946*.

There have been various developments during the last eighteen months but the position cannot yet be viewed with equanimity and this discussion provides an opportunity for an exchange of views on mycological education between mycologists of widely differing interests.

W. G. Keyworth. Mycology for the plant pathologist.

Experience is the greatest factor in producing a good Plant Pathologist, and it is inadvisable to lay too much stress on a detailed preliminary training. Nevertheless, it is obviously necessary for a pathologist to have as good a training as possible, and I propose to give a few of my own views on how this training should be devised.

At present most Plant Pathologists take a pure Science degree in Botany or an applied degree in Agriculture or Horticulture. In the pure Science degree, mycology is taught in much the same way as other aspects of Botany, with emphasis on the classification of the families which show features of taxonomic interest. In general the treatment of mycology in most University Degree courses is adequate for a preliminary training, but there must of necessity be many gaps. After taking his degree, the future pathologist often undertakes two years' research for a higher degree. This research is generally confined to a very restricted branch of the subject and, because of the demands it makes on the worker's time, there is little or no opportunity for broadening his general knowledge of mycology. The student may then become either an assistant in the Advisory Service or a research worker. If he becomes an adviser he obtains much practical knowledge in the course of his duties, and after a time may become reasonably well versed in his subject: if he becomes a research worker he usually continues to specialize and has little opportunity of learning the broader aspects of mycology.

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* These two reports are printed in the *Transactions*, xxxii, 100-112.

Many of the attributes of a good adviser cannot be learnt in any course, but must be inborn or be acquired by constant and sometimes somewhat painful contact with growers. On the mycological side, however, the main requirement seems to be a considerable skill in diagnosis, for which a wide knowledge of symptomatology is required. He has to be adept at identifying fungi, often from somewhat unsatisfactory material, and with the aid of books of reference which are not always very helpful. Having identified the cause, he must be able to indicate the best means of control, if any, and have a considerable practical knowledge of the difficulties which may be inherent therein.

The research worker has to be rather differently equipped. While generally specializing in some narrow branch of mycology, he must be capable of applying the results of other workers to his own problem. He must be skilled in the technique of studying a fungus *in vitro* and also in its host, be able to conduct careful inoculation experiments, and be conversant with the effects of environment on both pathogen and host.

Is the present system of training adequate for either of these workers? The degree training in mycology would not seem to help either very much, although necessary as a basis for further work. The subsequent concentration on a piece of research for a higher degree may be of value as a training in observation and experimental method, but it leaves the adviser with an incomplete general background and may make the research worker concentrate too much on special methods which he may not require thereafter.

How can these difficulties be overcome? I think both types of worker should continue their study of general mycology for some time after taking a degree. During such study, emphasis could be placed on pathogens, but not to the exclusion of all others, as these may often be encountered by pathologists in many branches of their work. All too often the Fungi Imperfecti are ignored because they have no life history or other taxonomic aspects of interest, but the group contains a very large number of important pathogens as well as many saprophytes normally encountered by pathologists. I suggest that in any scheme of training for pathologists thorough attention should be paid to this group, and to the methods by which it is classified. Included in the general mycological training there should be an adequate course on classification and taxonomy, with some insight into the problems of systematics. The student should also have ample opportunity of finding his way about the various keys which exist for identifying fungi, if only to learn the pitfalls that may beset him later. An opportunity should also be given for students to study the growth and physiology of a representative selection of pathogenic fungi, both *in vitro* and on their hosts, as well as the simple and more complex methods of isolating fungi.

After such preliminary training the courses for the two types of pathologist might diverge. The prospective adviser could, with great benefit, spend some time at an Advisory Centre to learn the practical problems, and to take an intensive course on the recognition in the field of diseases and their control. The research worker could concentrate on a study of research methods and technique in relation to a range of pathogens, supplemented by a small research programme.

Courses such as I have indicated are already given at one or two Universities in England, but they are generally undertaken during the degree courses and are therefore somewhat hurried. Post-graduate courses in pathology somewhat on these lines appear to be quite successful in America. I suggest, therefore, that consideration be given to the institution of a post-graduate training course for pathologists which would meet the requirements of the several types of worker, and which would incorporate the studies that experienced pathologists regard as of most value. Such a course might profitably lead to a recognized higher degree or a diploma in the subject, which would become accepted as a sound preliminary training.

F. D. ARMITAGE. Mycology for the industrial microbiologist.

There appears to be no course of instruction in applied mycology or any diploma in industrial mycology available to the staffs of industrial laboratories in this country. Such facilities are available in bacteriology, and the instruction includes descriptions of some of the common micro-fungi, chiefly to draw the attention of students to differences in morphology and nutritional requirements.

The microbiologist employed primarily to control the manufacture of a commodity produced by a fermentation process is usually a qualified biochemist who is prepared to make a special study of the physiology of the organisms of most interest to him by post-graduate study and research. On the other hand, anyone in an industrial laboratory who wishes to acquire a working knowledge of systematic mycology, in order to become familiar with the many kinds of micro-fungi which may spoil industrial products, has to rely almost entirely for mycological education on the information to be gained from reading the existing literature, and this is not an easy task, particularly without the guidance of one already conversant with mycology and mycological nomenclature. Indeed, to read the literature without such expert guidance tends to give the newcomer to mycology an impression of constant conflict between mycologists, who seem unable to agree for long on the family relationships of the specimens they so fondly foster.

This Society could therefore render great service to industry if it used its wide knowledge and influence to bring into being a school of mycology that would include, as part of its curriculum, tuition suitable for mycologists in industrial laboratories.

Apart from a general knowledge of the physiology of moulds and of their industrial uses and applications, the worker in the technical laboratory requires proper instruction in laboratory technique, with particular attention to the preparation and maintenance of culture collections. Methods of sampling technical products for mycological investigation should be standardized in principle, so that some measure of the degree of infection of a product can be accurately assessed. Instruction should also be given in experimental methods for comparing the value of industrial fungicides, for the inhibitors and methods used in phytopathology are often not applicable to industrial products. The correct use of inhibitors became a very important part of packaging research during the war years, particularly for materials going abroad and the export drive is now making the matter still more urgent. Some insight should be given into the morphology and cytology of the organisms to be studied as well as sufficient initial training to enable the industrial mycologist to place a fungus in its proper genus. It would also be helpful if the Society could organize a series of lectures later to be published and designed specifically for those beginning the study of micro-fungi.

I. Martin-Scott. Mycology for the medical practitioner.

Although fungi are less important as pathogens of man than of plants, their role as disease-producing agents was first recognized in animals and man. In 1835 (more than ten years before Berkeley's classic paper on Potato Blight) Bassi demonstrated that one organism could enter another and cause disease, when he showed experimentally that a fungus caused muscardine disease of silkworms. Four years later Schoenlein described the fungus of favus in man; and shortly afterwards, *Microsporum audouini*, a cause of ringworm of the scalp in children, was described by Gruby in France. These results stimulated research and speculation on the part fungi played in other human diseases, until later in the century the work of Pasteur, Koch and Lister emphasized the predominant role of bacteria in their causation. Subsequently, medical mycology received little attention until the eighteen nineties when the work and enthusiasm of Sabouraud in Paris led to similar investigations both in this country and elsewhere. These researches, chiefly on ringworm, were undertaken by medical men who had apparently few contacts with the non-medical mycologists of their day. In England, Colcott Fox, Blaxall and Adamson, among others, made notable contributions to medical mycology. Colcott Fox described and named *Trichophyton sulphureum*, which is still recognized as a 'good' species, and he and Blaxall also gave the first description of the cat ringworm fungus.

After 1895–1905, the heyday of British medical mycology, the subject fell into a decline and most doctors to-day are very ignorant of this interesting subject: an unhappy state that has come about because of the medical student's overcrowded curriculum and the poor facilities for post-graduate study. Medical mycology is not yet a subject in the undergraduate's already overloaded curriculum. During his six years' medical study ringworm infections are briefly outlined in the systematic lectures in dermatology: this amounts to about one hour's instruction. Thus the newly qualified doctor knows nothing of the microscopic mysteries of the mentagrophytes, or the perplexing problem of pleomorphism. He appreciates in a vague sort of way that ringworm may affect the scalps of children, and that the macerated grey soggy skin between the toes of his beach companion is due to a fungus.

If he takes up Public Health work he will receive a short course of lectures on medical mycology, and those medical men who practise dermatology find a knowledge of the dermatophytes imperative. Facilities for post-graduate study in mycology in this country are conspicuous by their paucity. The remedies are plainly obvious: the future medical student should be able to receive systematic instruction in mycology just as he receives instruction in bacteriology at the present time, and for the post-graduate, centres of mycology should be founded in the main cities where teaching could be undertaken and facilities offered to those interested in research.

These suggestions imply close collaboration between medical men and mycologists. 'Medical fungi' are much like other fungi, and it is rarely possible for a doctor to become familiar with more than the clinical aspects of the subject. The outstanding advances which have been made in the United States in the mycology and epidemiology of mycotic infections by the close collaboration of medical men and mycologists is a lesson we have yet to learn in this country.

J. M. B. KING. Mycology for the amateur.

In the field of phanerogamic botany amateurs can be divided into two classes—the one content to recognize and name the commoner flowers, and the other going much more deeply into species, varieties and forms and having some knowledge of plant physiology. These divisions seem hardly to exist in the mycological world. The extreme difficulty of identifying fungi, together with the absence of suitable books and practical help, soon discourages the faint-hearted and only the more determined enthusiast survives, the practical result being a small but select group that takes its mycology seriously. Such a group should receive every help and encouragement, but it would be wrong to ignore the needs of the weaker groups.

Briefly, the needs of the amateur are literature and personal contact. Even in normal times the shortage of suitable books has been a serious handicap to the student of fungi, and to-day recourse must usually be had to second-hand bookshops where prolonged search may occasionally unearth a copy of Massee, Cooke or Berkeley. Even so, such works are long out of date, often inaccurate and seldom include microscopic data such as spore size, cystidial characteristics and so on. Our classic stand-by for the larger fungi—Rea's *British Basidiomycetes*—is unobtainable and unlikely to be reprinted. One of the few books available to-day is Ramsbottom's *Handbook of the Larger British Fungi*. Unfortunately this contains little microscopic data and no coloured illustrations, both of which are essential for successful diagnosis. Certain foreign books can be obtained, notably Lange's *Flora Agaricina Danica*, but they are usually expensive and difficult to obtain. Under literature three important requirements are glossaries, colour charts and keys. Glossaries should form a part of nearly every scientific work, and certainly every work designed for students, for if the reader knows less of the subject than the author, then it may be assumed that he also knows less of the terms. Authors seem often to overlook the fact that though a student may be familiar with general mycological terms, he may be ignorant of the special terms used. Let us too, have liberal translations of Latin and Greek terms. We cannot all be classical scholars and an immense amount of time can be wasted in searching through dictionaries for meanings. There is every reason for generic and specific names to be in Latin, though in an English work English equivalents should be included, but I can see no reason for Latin and Greek in other respects. Diagnostic keys are favourite places to sprinkle with Latin tags. For 'carnosae', 'coloratae', 'albatae', 'megasporae', 'mesosporae', 'stenosporae', would it not be just as easy to say 'fleshy', 'coloured', 'whitish', 'large spores', 'medium spores' or 'narrow spores'? Even Lange's masterpiece, a work obviously designed for English-speaking peoples, is irritatingly profuse in such terms.

Closely following the need for new books with good glossaries comes the need for good colour charts. It is often extremely difficult to describe the colour of a fungus and the difficulty is added to by the use of such inexactitudes as 'alutaceous', 'isabelline' and 'ochraceous'. For my part I never know whether an author means red or yellow ochre when he speaks of 'ochraceous' and as for the tans and tawnys and livids, they cover a vast range of tones. Would it not be possible for the British Mycological Society to publish in the *Transactions* a standard colour chart? What a boon it would be if it could be decided once and for all what tint was intended by 'ochraceous'; it would matter little whether this was red, yellow or even blue or green as long as we all understood the same thing.

From colour charts we come naturally to keys. Here there is immense scope for improvement. The simple dichotomous systems used so successfully with flowering plants are less easily applied to fungi and we still await the perfect system, but meanwhile the task of identifying fungi is not made easier by the constant shifting of species from one genus to another, or the creation of new genera in the ceaseless attempt to find a natural classification. As an example of this I may mention *Armillaria mucida*. This species has now been placed by Lange in *Collybia* and becomes *Collybia mucida*. In making this change one of the fundamentals of every known key is shattered—the presence or absence of a ring. How can simple and usable keys be evolved when exceptions to their very foundations have to be made? I see only one solution to the problem—two systems of classification. One as straightforward and simple as possible, designed for the express purpose of identifying fungi, the other—the natural system—for pure science. I see little objection to such a duality, for once a specimen had been diagnosed by the simple system it could readily be referred to its position in the natural system if necessary. In devising any revised system of classification a very necessary first step is a clean sweep of the numerous ghost species now cluttering up practically every text-book. Many fungi given specific rank could probably be reduced to varieties or forms and by thus thinning out or condensing the present long lists of species, which most mycologists have never seen and probably never will see, the task of diagnosis would be made much simpler.

Of even greater benefit to the amateur would be the allocation to his special needs of a few pages in each issue of the *Transactions*; short but authoritative articles on the elements of mycology—e.g. cytology, taxonomy, classification, generic notes—would be extremely welcome and go some distance to meet the shortage of up-to-date literature.

The other important requirement of the amateur is personal contact. In the study of any subject books in themselves are insufficient; there comes a time when practical guidance and help is necessary. Those of us who study the larger fungi well remember the days when returning from the woods with a basket of specimens we attempted the hopeless task of reducing the confusion to order. It soon became apparent that no advance could be made without the finger of experience to point the way. Forays are one form of personal contact: they are an essential and vital part of the Society's activities, providing an opportunity not only to meet experienced mycologists but to benefit from that experience on the spot. I admire the careful way in which these forays are conducted and particularly the manner in which the specimens are laid out and labelled at the end of the day: and I know of no other Society that does this.

Forays apart there exists only one other opportunity for personal contact and that is in the meetings of the Society. These meetings are delightful in character, and provide a blend of science and sociability. As an amateur, however, may I make one criticism: almost invariably these meetings deal with micro-fungi and related matter of an advanced character, and I often feel how much I would like to hear the same speakers lecture on the larger fungi or on taxonomy, classification, cytology or micro-fungi from a humble aspect. The amateur admittedly forms but a small part of the Society, but the experts may not be experts in every branch of mycology and an agaricologist would doubtless enjoy a simple talk on micro-fungi and vice versa. Personal contact for the amateur takes the place of the lecturer and demonstrator of the professional student and I know this society will never express towards its humbler members the sentiment I heard recently in another place 'Oh, we can't be bothered with the rabble'.

C. G. C. CHESTERS. Mycology in the Universities.

Orthodoxy. Mycology has been, and still is, regarded as a part of the curriculum of a botany course in a University. In most British Universities a student who desires to specialize in mycology arrives at his goal by way of an honours course in botany followed by graduate research and with the ultimate qualification of a M.Sc. or of a Ph.D. in botany. From first to last the word mycology does not enter into the documented qualifications with which he is credited, except in so far as testimonials or references from the department of the University concerned may indicate the line of specialization which he has followed, and except in so far as the particular department has an external reputation for that type of specialization.

This is the accepted course and it will continue to be accepted in a number of Universities. It can be justified and it has many points of value. We believe in Britain that specialization at the undergraduate level is not desirable; that we should not train for professional careers as such; that we should provide a liberal education. We have inherited the view that fungi are members of the vegetable kingdom and they are usually closely associated with the algae in the phylum Thallophyta. This acceptance of a point of view has resulted in potential mycologists being more or less well versed in the field of botany, perhaps in the whole field of botany, and it has resulted in many advantages for the mycological student, as a mycologist or as a would-be plant pathologist. He has been required to know plants and this should have been of immense value in the study of organisms which frequently live on plant substrates. The knowledge he has acquired in plant taxonomy, anatomy and physiology can all be brought to bear on his future selected topic of study. This line of study has a tradition behind it and much can be made of its underlying principles.

As an initial point of discussion I believe that the fungi should be removed from the Thallophyta and should be treated quite independently of the algae both in taxonomic position and time of appearance in the syllabus. The following outline appears to me to be a desirable method of treating the fungi within the syllabus of a botany honours school. During the intermediate course (if such a course continues to be given in the Universities) the student should encounter the fungi principally from the standpoint of life-cycle studies in relation to their importance to man with sufficient emphasis on their structure, function, reproduction, genetics and classification to impress on him their biological peculiarities and their importance both to the life of the green plant and to the animal kingdom. In position, such a treatment should follow a résumé of the phyla of the plant kingdom. Passing to the honours school the student is destined to spend three years in the study of plants, and I am presuming that during the first two of these years he will cover the whole of the subject-matter in a general way, and that during the last year he will return to selected topics for discussion in greater detail. In such a course the precise location of his studies of fungi becomes important. I believe it should follow his consideration of the physiology and the anatomy of the green plant and should therefore come within the second year of that course. The subject-matter should be based essentially on the study of the taxonomy of the fungi, and should deal with the separation of the larger units of the class as illustrated by named species, studied as far as possible in the fresh condition on their characteristic substrates. Every effort should be made to give a good, reasoned account of the class, bringing into the scheme relevant facts on the physiology and the genetics of species. Students should be encouraged to collect and to study the fungi in the field and should be led to realize that fungi are to be found in specific localities growing under specific conditions. When the final year is reached the curriculum of each student may be varied according to certain selected interests. The student is now able to devote each day to botanical studies and at this stage I should introduce a short course of bacteriology covering the principles and the technique of this subject. This is followed by studies of the physiology of fungi, of the detailed taxonomy of specific families as exercises in the application of criteria for the recognition of species, of the genetics of fungi, of the economic importance of fungi to mankind and of the principles of isolation, cultivation and preservation of fungi. The student might be required to attend lectures elsewhere in the department on plant pathology and to spend a week in field work dealing with the collection and identification of fungi. A short investigation of a specific topic or the discussion of a particular field of literature might well be introduced.

Graduate studies can be built on such a foundation and I should plead here the importance of graduate studies in formal mycology as a further course for certain professional careers rather than the immediate undertaking of research projects in mycology. I am certain that there is a necessary place for graduate diploma courses in mycology in the training of the student who has passed through the curriculum of an honours school of botany, and such advanced formal studies are stimulating both for student and teacher. The suggestion that a Master's degree in mycology might well be a first research degree has been made in this Society's report on the Teaching of Mycology and I believe this to be an excellent step in the training of a professional mycologist.

Heterodoxy. Why not break with tradition and come right out into the open with the declaration that mycology is a science of equal standing with bacteriology and, for that matter, with botany, and provide a course for an honours school in mycology? The background of the course, after intermediate or its equivalent had been passed, would be a one year subsidiary course in chemistry (or botany), along with two session courses in botany (or chemistry) and in mycology, followed by a final honours course in mycology. The outline of the initial mycological course would consist of a good grounding in bacteriology and in the physiology of fungi, followed by detailed consideration of the taxonomy of the groups of fungi. The final honours year would be devoted to intensive study of fungi both as individual organisms and as agents causing disease and as organisms capable of being induced to perform various organic syntheses. During this year organized field studies would play a large part in the course. It is not an impossible dream and, if we are to play our part in the world to-day, I submit that it must become a reality at some centre in Britain.

Field work. It may not always be possible to secure constant access to a reasonably private location near a University to permit continued and progressive study of the fungal flora by successive years of students, but there can be no doubt that such a system of field work is highly desirable. Similarly, an annual departmental foray gives the essential insight into field studies without which a mycological education is incomplete. As a complementary study to the fungi in the field the development of a teaching herbarium with well-documented collections of selected species within particular genera is an essential equipment of a mycological department. Such a herbarium should constantly extend and furnish a progressively expanding court of reference for the training in systematic mycology.

Conclusions. Whether a student be trained in the orthodox or the heterodox system outlined above there is one basic condition which must be fully satisfied. The study of mycology is rooted in a knowledge of the living fungus in its natural habitat, and by every available means—collecting excursions and forays of this Society—the student of mycology must be initiated to and raised on a study of the fungus in its natural association with the plant and animal life of the world.

L. M. J. KRAMER. Mycology in relation to schools.

Clearly the work of schools is predominantly that of education. Nevertheless, practically all secondary grammar schools and the newly set up modern schools make some provision for general science, while some, fortunately placed in point of situation, staff or facilities, emphasize biology or nature study and field work. Therefore some attention to mycology is possible, but obviously it can only be by passing reference or by elementary study of a few common moulds or higher basidiomycetes.

Beyond the standard of the School Certificate Examination much more time may be devoted to biology by candidates for Higher School Certificate or University Entrance Scholarship Examinations in Grammar Schools. Such pupils are the basic source of professional biologists, and their minds might suitably be directed to the implications of mycology and the possible future openings for those able to undertake the long training.

From the foregoing very brief statement of the present position certain suggestions arise. The advance of the research front cannot normally be followed adequately by school teachers, so that a method of informing them of recent trends, and of the need for change of emphasis of syllabuses, is called for. A medium for this exists, namely the *School Science Review*, the official organ of the Science Masters' Association. Articles at intervals on mycological matters would meet the need.

Advice on openings for mycologists and on suitable University and other centres for training could also be communicated to the *School Science Review*. The possible employment of non-graduates in a technical capacity in mycological laboratories should be borne in mind in preparing such communications.

For schools situated in large cities, where field work is extremely difficult, simple mycological work in laboratories is quite possible, and can serve to illustrate a number of basic principles of scientific experimentation. Notes on suitable work might perhaps be printed in the Society's *Transactions*.

The meeting concluded with a review by C. T. Ingold of the papers and subsequent discussion, in which he emphasized the important place that the Society holds in relation to mycological education and to mycological work generally, and in which he made suggestions for future progress in the different aspects of the subject.

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